IMP. INST. ENTOM.
— LIBRARY —
No. 7738

P.R.F.

ICAR. 17 VII.
800.

Vol. VII 1937

THE INDIAN JOURNAL OF AGRICULTURAL SCIENCE

Issued under the Authority
of
The Imperial Council of Agricultural Research

Annual subscription *Price per part*

Rs. 15 or 24s. *Rs. 3 or 5s. 3d.*

Published by the Manager of Publications, Delhi.
Printed by the Manager, Government of India Press, New Delhi
1938.

Price annas 6 or 7d.

IMP. INST. ENT.
— LIBRARY —
28 NOV 1938
SERIAL As.60B
SEPARATE

List of Agents in India from whom Government of India Publications are available.

(a) PROVINCIAL GOVERNMENT BOOK DEPOTS.

MADRAS :—Superintendent, Government Press, Mount Road, Madras.
BOMBAY :—Superintendent, Government Printing and Stationery, Queen's Road, Bombay.
SIND :—Manager, Sind Government Book Depot and Record Office, Karachi (Sadar).
UNITED PROVINCES :—Superintendent, Printing and Stationery, U. P., Allahabad.
PUNJAB :—Superintendent, Government Printing, Punjab, Lahore.
CENTRAL PROVINCES :—Superintendent, Government Printing, Central Provinces, Nagpur.
ASSAM :—Superintendent, Assam Secretariat Press, Shillong.
BIHAR :—Superintendent, Government Printing, P. O. Gulzarbagh, Patna.
NORTH-WEST FRONTIER PROVINCE :—Manager, Government Printing and Stationery, Peshawar.
ORISSA :—Press Officer, Secretariat, Cuttack.

(b) PRIVATE BOOK-SELLERS

Advani & Co., The Mall, Cawnpore.
Aero Stores, Karachi.*
Banthiya & Co., Ltd., Station Road, Ajmer.
Bengal Flying Club, Dum Dum Cantt.*
Bhatia Book Depot, Saddar Bazar, Ranikhet.
Bhawnani & Sons, New Delhi.
Bombay Book Depot, Charni Road, Girgaon, Bombay.
Book Company, Calcutta.
Booklover's Resort, Taikad, Trivandrum, South India.
Burma Book Club, Ltd., Rangoon.
Butterworth & Co. (India), Ltd., Calcutta.
Cambridge Book Co., Booksellers, New Dak Bungalow Road, Patna.
Careers, Mohini Road, Lahore.
Chatterjee & Co., 3, Bacharam Chatterjee Lane, Calcutta.
Chukervertty, Chatterjee & Co., Ltd., 13, College Square, Calcutta.
City Book Club, 98, Phayre Street, Rangoon.
Das Gupta & Co., 54/3, College Street, Calcutta.
Dastane Brothers, Home Service, 456, Raviwar Peth, Poona 2.
Delhi and U. P. Flying Club, Ltd., Delhi.*
Educational Book Depot, Mahal Road, Nagpur.
English Book Depot, Ferozepore.
English Book Depot, Taj Road, Agra.
English Bookstall, Karachi.
English Book Store, Abbottabad, N.-W. F. P.
Faqir Chand Marwah, Peshawar Cantonment.
Gautama Brothers & Co., Ltd., Meston Road, Cawnpore.
Higginbothams, Madras.
Hindu Library, 137/F, Balaram De Street, Calcutta.
H. L. College of Commerce, Co-operative Stores, Ltd., Ahmedabad.
House of Knowledge, Booksellers and Publishers, Palliagraharam P. O., Tanjore.
Hyderabad Book Depot, Chaderghat, Hyderabad (Deccan)
Ideal Book Depot, Rajpur Road, Dehra Dun.
Imperial Book Depot and Press, near Jama Masjid (Machhliwalan), Delhi.
Imperial Publishing Coy., Lahore.
Indian Army Book Depot, Dayalbagh, Agra.
Indian Army Book Depot, Daryaganj, Delhi.
Indian Book Shop, Benares City.
Indian School Supply Depot, Central Avenue, South, P. O. Dharamtala, Calcutta.
Insurance Publicity Co., Ltd., Lahore.
International Book Service, Poona 4.
Jaina & Bros., Mori Gate, Delhi, and Connaught Place, New Delhi, Messrs. J. M.
Kamala Book Depot, 15, College Square, Calcutta.
Keale & Co., 65, Britto Road, Karachi (Sadar).
Keshao Book Stall, Khadibazar, Belgaum.
Kitabistan, 17-A, City Road, Allahabad.
Krishnaswami & Co., Teppakulam P. O., Trichinopoly Fort, Messrs. S.
Lahiri & Co., Calcutta, Messrs. S. K.
Local Self-Govt. Institute, Bombay.
London Book Co. (India), Arbab Road, Peshawar, Murree, Nowshera, Rawalpindi.
Mackwin & Co., Booksellers, Stationers and News Agents, Inverarity Road, off Esphenstone Street, Karachi Sadar.
Malhotra & Co., Post Box No. 94, Lahore, Messrs. U. P.
Malik & Sons, Sialkot City.
Mathur & Co., Messrs. B. S., Chatur-Vilas, Paota, Civil Lines, Jodhpur (Rajputana).
Minerva Book Shop, Anarkali Street, Lahore.
Modern Book Depot, Bazar Road, Sialkot Cantonment.
Mohanlal Dossabhai Shah, Rajkot.
Mohendra Bros. Lashkar, Gwalior State, Messrs.
Nandkishore & Bros., Chowk, Benares City.
New Book Co., "Kitab Mahal", 192, Hornby Road, Bombay.
Newman & Co., Ltd., Calcutta, Messrs. W.
Oxford Book and Stationery Company, Delhi, Lahore, Simla, Meerut and Calcutta.
Parikh & Co., Baroda, Messrs. B.
Pioneer Book Supply Co., 20, Shib Narayan Das Lane, Calcutta and 219, Cloth Market, Delhi.
Popular Book Depot, Grant Road, Bombay.
Punjab Religious Book Society, Lahore.
Raghunath Prasad & Sons, Patna City.
Rama Krishna & Sons, Booksellers, Anarkali, Lahore.
Ram Krishna Bros., Opposite Bishrambag, Poona City.
Ram Narain Lal, Katra, Allahabad.
Ramesh Book Depot and Stationery Mart, Kashmere Gate, Delhi.
Ray & Sons, 43, K. & L. Edwardes Road, Rawalpindi, Murree and Peshawar, Messrs. J.
Roy Chowdhury & Co., 72, Harrison Road, Calcutta, Messrs. N. M.
Saraswati Book Depot, 15, Lady Hardinge Road, New Delhi.
Sarcar & Sons, 15, College Square, Calcutta, Messrs M. C.
Sarkar & Co., Ltd., 18, Shama Charan De Street, and 6 Hastings Street, Calcutta, Messrs. P. C.
Sharada Mandir Ltd., Nai Sarak, Delhi.
Standard Bookstall, Karachi.
Standard Bookstall, Quetta.
Standard Book Depot, Lahore, Dalhousie and Delhi.
Standard Law Book Society, 69, Harrison Road, Calcutta.
Tara & Sons, Razmak (India), Messrs. B. S.
Taraporevala Sons & Co., Bombay, Messrs. D. B.
Thacker & Co., Ltd., Bombay.
Thacker, Spink & Co., Ltd., Calcutta and Simla.
Tripathi & Co., Booksellers, Princes Street, Kalbadevi Road, Bombay, Messrs. N. M.
Uberoy, J. C., Journalist, Printer and Publisher, Jaycu House, Alexandra Road, Ambala.
Universal Book Depot, 58, Hazratganj, Lucknow.
University Book Agency, Kacheri Road, Lahore.
Upper India Publishing House, Ltd., Literature Palace, Ammuddaula Park, Lucknow.
Varadachary & Co., Madras, Messrs. P.
Venkatasubban, A., Law Bookseller, Vellore.
Wheeler & Co., Allahabad, Calcutta and Bombay, Messrs. A. H.
Young Man & Co. (Regd.), Egerton Road, Delhi.

*Agent for publications on Aviation only.

CONTENTS OF VOLUME VII

Part I (February, 1937)

Original articles—

		Page
Studies in Crop Ecology, I. The Composition of the Cotton Crops of Central India and Rajputana (with one text-figure)	*J. B. Hutchinson and R. L. M. Ghose*	1
Variation in the Characters of Cotton in relation to the Position of Bolls on the Plant (with one text-figure)	*K. R. Sen, D.Sc. and Mohammad Afzal, B.Sc. (Agri.), A.I.C.T.A.*	35
Studies in Indian Barleys, IV. The Inheritance of some Anatomical Characters Responsible for Lodging and of some Ear-head Characters in an Inter-specific Cross between two Pusa Barleys (with Plates I-III and four text-figures)	*R. D. Bose, B.Sc.; M.A. Aziz, B.Sc. (Agri.) and M. P. Bhatnagar, B.Sc. (Hons.)*	48
Studies in the Water Relations of Rice, I. Effect of Watering on the Rate of Growth and Yield of Four Varieties of Rice (with Plate IV and seven text-figures)	*P. K. Sen, M.Sc. (Cal.), Ph.D. (Lond.), D.I.C.*	89
Heterosis in Rice	*B. S. Kadam, G. G. Patil and V. K. Patankar*	118
Inheritance of Inner Glume Colour in Rice (with Plates V and VI)	*S. K. Mitra, I.A.S. and P. M. Ganguli*	126
Inheritance of Sheathed Ear in Rice (with Plates VII-IX)	*R. L. Sethi, M.Sc., B.Sc. (Edin.), I.A.S.; B. L. Sethi, M.Sc., Ph.D. (Wales) and T. R. Mehta, B.Sc.*	134
Preliminary Experiments on the Mass-production of *Trichogramma* Parasites for Control against Sugarcane Borers in Mysore (with Plates X and XI)	*T. V. Subramaniam*	149
Importance of the Study of Insect Ecology in Applied Entomology (with three text-figures)	*Taskhir Ahmad, Ph.D. (Cantab.)*	156

		PAGE
The Cold Storage of Nagpur Oranges (*Citrus aurantium*)	*G. S. Cheema, D.Sc., I.A.S.; D. V. Karmarkar, Ph.D. and B. M. Joshi, M.Sc.*	168
Ontogenetic Drifts in the Physiology and Chemistry of Tropical Fruits under Orchard Conditions (with six text-figures)	*B. N. Singh, D.Sc.; P. V. V. Seshagiri, M.Sc. and S. S. Gupta, M.Sc.*	176
Statistical Notes for Agricultural Workers :—		
No. 22.—A Statistical Study of the Yields of the Permanent Manurials, Pusa	*T. V. G. Menon, B.A., B.Sc. (Agri.), Assoc. I.A.R.I. and S. S. Bose, M.Sc.*	193
No. 23.—A Statistical Study of the Deterioration of Yields of the Permanent Manurials, Pusa	*S. S. Bose, M.Sc. and T. V. G. Menon, B.A., B.Sc. (Agri.), Assoc. I.A.R.I.*	205

Abstract—

Analysis of *Crotalaria juncea* with special reference to its Use in Green-manuring and Fibre Production	..	214

Part II (April, 1937)

Original articles—

The Classification of the Cottons of Asia and Africa (with Plates XII-XIV and twenty-eight text-figures)	*J. B. Hutchinson and R. L. M. Ghose*	233
Studies on the Root-rot Disease of Cotton in the Punjab, III. The Effect of some Physical and Chemical Factors on Sclerotia Formation (with three text-figures)	*R. Sahai Vasudeva, B.Sc., Ph.D. (Lond.), D.I.C.*	259
A Comparative Study of Species of *Septoria* occurring on Wheat (with Plates XV-XVII and three text-figures)	*Jai Chand Luthra, M.Sc., D.I.C. (Lond.); Abdus Sattar, B.Sc. (Ag.), Assoc. I.A.R.I., Ph.D. (Lond.), D.I.C. (Lond.) and M. Abdul Ghani, M.Sc. (Ag.)*	271
Molasses as Manure in the United Provinces	*H. N. Batham, M.A.; R. L. Sethi, M.A., B.Sc. and L. S. Nigam, L.Ag.*	291

		PAGE
Studies on *Schistocerca gregaria* Forsk., IX. Some Observations on the Histology of the Blood of the Desert Locust (with Plates XVIII and XIX)	*C. B. Mathur, M.Sc. (Luck.) and B. N. Soni, B.Sc. (Edin.)*	317
Plant-Growth in relation to Hydrogen-ion Changes in its Medium (with Plates XX and XXI and one text-figure)	*B. N. Singh, D.Sc. and G. P. Mitra, M.Sc.*	327

Notes—

Notification	..	349
The Fourth International Grassland Congress	..	349

Book Notices—

The Experimental Production of Haploids and Polyploids	..	351
The South American Potatoes and their Breeding Value	..	351

Part III (June, 1937)

Original articles—

Types of Tobacco Leaf-curl in Northern India (with Plates XXII-XXXI and five text-figures)	*B. P. Pal, M.Sc., Ph.D. (Cantab.), F.L.S. and R. K. Tandon, M.Sc.*	363
The Pulse Beetles (*Bruchidae*) of Burma (with Plates XXXII and XXXIII)	*C. C. Ghosh, B.A., F.R.E.S.*	395
Studies in *Fusarium* Wilt of Sann-hemp, I. The Physiology and Biology of *Fusarium vasinfectum* Atk. (with one text-figure)	*B. N. Uppal and N. T. Kulkarni*	413
An Anthracnose Disease of Sann-hemp (with Plates XXXIV-XXXVII)	*M. Mitra, Ph.D. (Lond.), D.Sc. (Lond.), D.I.C.*	443
A Genetical Study of Roots in Relation to Disease-resistance in Cotton	*V. G. Panse and A. F. Patel*	451
Studies on the Stinking Smut or Bunt of Wheat in India (with two text-figures)	*M. Mitra, Ph.D. (Lond.), D.Sc. (Lond.), D.I.C.*	459
The Effect of different Cations on the Living Protoplasm of the Root Hair of *Azolla pinnata* (with Plates XXXVIII and XXXIX and one text-figure)	*B. Sen* . . .	479
Motes in Cotton, II. Punjab *Desi* Cottons .	*Mohammad Afzal* .	487
The Development of the Microspores in *Trichosanthes dioica* Roxb. (with Plates XL-XLIII and five text-figures)	*I. Banerji and M. C. Das*	497

PAGE

ON THE *PYRILLA* PEST OF SUGARCANE IN INDIA (WITH PLATES XLIV-XLVI) — *H. S. Pruthi, M.Sc., Ph.D. (Cantab.), F.N.I.* — 511

SUGARCANE-BAMBOO HYBRIDS (WITH PLATES XLVII AND XLVIII) — *T. S. Venkatraman, C.I.E., B.A., I.A.S.* — 513

Reviews—

AN OUTLINE OF CYTOLOGICAL TECHNIQUE FOR PLANT BREEDERS .. 515

VEGETATIVE PROPAGATION OF TROPICAL AND SUB-TROPICAL FRUITS .. 517

HORTICULTURAL ASPECTS OF WOOLLY APHIS CONTROL TOGETHER WITH A SURVEY OF THE LITERATURE .. 518

EEN BIJDRAGE TOT DEKENNIS OMTRENT HET BLOEIEN EN HET VRUCHTDRAGENDE VERMOGEN VAN DEN MANGGA (*Mangifera indica* L.). (A CONTRIBUTION TO THE KNOWLEDGE OF THE FLOWERING AND FRUITING HABITS OF THE MANGO TREE, *Mangifera indica* L.) .. 519

Part IV (August, 1937)

Original articles—

STUDIES IN PLANT BREEDING TECHNIQUE, II. THE DESIGN OF FIELD TESTS OF PLANT BREEDING MATERIAL (WITH FIVE TEXT-FIGURES) — *J. B. Hutchinson and V. G. Panse* — 531

DISEASES OF THE TEA BUSH IN THE KANGRA VALLEY, PUNJAB, I (WITH PLATES XLIX AND L) — *H. Chaudhuri ; V. S. Kapur ; K. L. Bhatia and J. S. Anand* — 565

STUDIES ON THE ROOT-ROT DISEASE OF COTTON IN THE PUNJAB, IV. THE EFFECT OF CERTAIN FACTORS INFLUENCING INCIDENCE OF THE DISEASE (WITH FOUR TEXT-FIGURES) — *R. Sahai Vasudeva, B.Sc., Ph.D. (Lond.), D.I.C. (Lond.)* — 575

AWNEDNESS AND ITS INHERITANCE IN RICE (WITH PLATE LI) — *R. L. Sethi, M.Sc., B.Sc. (Edin.), I.A.S. ; B. L. Sethi, M.Sc., Ph.D. (Wales) and T. R. Mehta, B.Sc.* — 589

SAMPLING OF SUGARCANE FOR CHEMICAL ANALYSIS, I (WITH FOUR TEXT-FIGURES) — *Ramji Narain, Ph.D., D.Sc. and Azmat Singh, L.Ag.* — 601

RELATIVE SUSCEPTIBILITY OF SOME WILD AND CULTIVATED POTATO VARIETIES TO AN EPIDEMIC OF LATE-BLIGHT AT SIMLA IN 1936 (WITH PLATE LII) — *B. B. Mundkur ; B. P. Pal and Pushkar Nath* — 627

PAGE

The Grape-vine Thrips (*Rhipiphorothrips cruentatus* Hood) (Thripidae : Terebrantia : Thysanoptera) (with Plates LIII-LV) — *Khan A. Rahman, B.Sc. (Edin.), Ph.D. (Cantab.) and Nand Kishore Bhardwaj M.Sc. (Pb.)* 633

Germination of Fungal Spores in relation to Atmospheric Humidity — *S. Chowdhury* . 653

Entomological Investigations on the Leaf-curl Disease of Tobacco in North Bihar (with Plates LVI-LVIII)— *Hem Singh Pruthi, M.Sc., Ph.D. (Cantab.) F.N.I. and C. K. Samuel, B.Sc.* 659

I. Transmission Experiments with some Suspected Insect Vectors.

II. An Alternative Host of the Virus and the Insect Transmitter

Abstract—

Soil Deterioration in the Canal-irrigated Areas of the Punjab. .. 671

Note 673

Part V (October, 1937)

Original articles—

Cytological Studies in Paddy Varieties (with Plates LIX-LXI) — *B. L. Sethi, M.Sc., Ph.D. (Wales)* 687

A Note on the Cyto-genetics of *Ricinus communis* L. (with six text-figures) — *R. H. Richharia, Ph.D.* 707

Observations on the Milling of Rice in the United Provinces (with Plates LXII and LXIII and two text-figures) — *R. L. Sethi, M.Sc., B.Sc. (Edin.), I. A. S.; B. L. Sethi, M.Sc., Ph.D. (Wales); T. R. Mehta, B.Sc. and P. S. Gupta, M.Sc. (Luck.), M.Sc. (Lond.)* 713

A Note on a few Experimental Observations in the Rice Research Station, Berhampur (Madras) — *M. B. V. Narasinga Rao, B.A., B.Sc. (Ag.), Assoc. I.A.R.I.* 733

Agricultural Meteorology : The Prediction of the Minimum Temperature on Clear Days at Selected Stations in India (with seven text-figures) — *M. Narasimhan, M.A. and L. A. Ramdas, M.A., Ph.D.* 745

		PAGE
STUDIES ON THE DISSOCIATION OF *BACILLUS CEREUS*, AN ORGANISM ASSOCIATED WITH PLANTS AFFECTED WITH MOSAIC DISEASE (WITH PLATES LXIV-LXVI)	*N. V. Joshi and S. C. Dutt*	763
THE BIRD ENEMIES OF THE COTTON LEAF-ROLLER (*SYLEPTA DEROGATA* FB.) AT KHANEWAL, MULTAN (PUNJAB)	*M. Afzal Husain, M.A. (Cantab.), I.A.S. and Hem Raj Bhalla, B.Sc. (Ag.)*	785
A NEW DISEASE OF *ELEUSINE CORACANA* GAERTN. (WITH PLATES LXVII AND LXVIII)	*P. R. Mehta and S. C. Chakravarty*	793
A NEW CECIDOMYID PEST OF LINSEED IN INDIA (WITH PLATES LXIX AND LXX)	*Hem Singh Pruthi, M.Sc., Ph.D. (Cantab.), F.N.I. and H. L. Bhatia, M.Sc.*	797

Reviews—

DRILLING MUD: ITS MANUFACTURE AND TESTING	..	809
ANNUAL REVIEW OF BIOCHEMICAL AND ALLIED RESEARCH IN INDIA, VOL. VII, 1936	..	810
THE PLANT DISEASES OF GREAT BRITAIN	..	810
HERBAGE ABSTRACTS, VOL. 7, NO. 1, MARCH 1937	..	810

Part VI (December, 1937)

Original articles—

OBSERVATIONS ON PHYLLODY OF SESAMUM IN BURMA (WITH THREE TEXT-FIGURES)	*D. Rhind, F.D. Odell and U Thet Su*	823
STUDIES IN INDIAN OIL-SEEDS, VI. SOME CORRELATIONS BETWEEN OIL-CONTENT AND OTHER CHARACTERS IN THE PUSA LINSEED HYBRIDS (WITH PLATE LXXI)	*R. B. Deshpande, M.Ag. (Bom.) and A. K. Mallik, M.Sc. (Bot.), B.Sc. (Ag.).*	841
BREEDING INVESTIGATIONS IN SOME OF THE OLEIFEROUS *BRASSICAE* OF THE PUNJAB (WITH PLATE LXXII)	*Ali Mohammad, M.Sc. (Agri.), L.Ag. and Sawan Mal Sikka, B.Sc. (Agri.), Assoc. I.A.R.I.*	849
COLOURS IN THE RICE GRAIN (WITH PLATES LXXIII-LXXVI)	*K. Ramiah, M.Sc., Dip. Agri. (Cantab.), L.Ag., and C. Rajasekhara Mudaliar, M.A.*	863

		PAGE
A NOTE ON TWO NEW GENES AFFECTING ANTHOCYANIN PIGMENTATION IN ASIATIC COTTONS	*J. B. Hutchinson and R. L. M. Ghose*	873
EFFECT OF FARMYARD MANURE ON FIBRE-CHARACTERS OF COTTON (WITH SEVEN TEXT-FIGURES)	*H. R. Nayak* . .	877
THE INFLUENCE OF NON-NITRIFYING ORGANISMS ON NITRIFICATION	*S. V. Desai, D.Sc. Ph.D., D.I.C. and Fazal-ud-Din, B.Sc.*	895
THE BLACK BEETLE (*ALISSONOTUM IMPRESSICOLLE* ARR.): A PEST OF SUGARCANE IN MYITKYINA DISTRICT IN NORTHERN BURMA (WITH PLATES LXXVII AND LXXVIII AND FIVE TEXT-FIGURES)	*C. C. Ghosh, B.A., F.R.E.S.*	907

Mycological Note—

UROMYCES RUMICIS (SCHUM.) WINTER: A NEW RECORD FOR INDIA	*T. B. Lal, B.Sc. (Agri.)*	933

Note—

LENGTH REGULARITY AND PERCENTAGE OF SHORT FIBRES IN COTTONS	..	935

Review—

PROCEEDINGS OF THE ASSOCIATION OF APPLIED BIOLOGISTS, OCTOBER 1936	..	937

INDEX TO VOLUME VII

A

Page

Abdul Ghani, M. (Luthra, J. C. and)—Species of *Septoria* occurring on wheat . . . 271

Abdus Sattar (Luthra, J. C. and)—Species of *Septoria* occurring on wheat . . . 271

Africa and Asia, classification of the cottons of (Hutchinson, J. B. and Ghose, R.L.M.) . . . 233

Afzal Husain, M. and Bhalla, Hem Raj.—The Bird Enemies of the Cotton Leaf-roller (*Sylepta derogata* Fb.) at Khanewal, Multan (Punjab) . . . 785

Afzal, Mohammad.—" Motes in Cotton, II. Punjab *Desi* Cottons " . . . 487

Afzal, M. (Sen, K. R. and)—Variation in the characters of cotton in relation to the position of bolls on the plant . . . 35

Agricultural meteorology (Narasimhan, M. and Ramdas, L. A.) . . . 745

Ahmad, T.—" Importance of the Study of Insect Ecology in Applied Entomology " . . . 156

Ali Mohammad and Sikka, Sawan Mal.—" Breeding Investigations in some of the Oleiferous *Brassicae* of the Punjab " . . . 849

Alissonotum impressicolle (Black beetle), a pest of sugarcane in Myitkyina district (Ghosh, C. C.) . . . 907

Alkalisation, and dispersion coefficient in deteriorated soil, relation between (McKenzie Taylor, E. *et al.*) . . . 671

Anand, J. S. (Chaudhuri, H. and)—Diseases of tea bush in the Kangra Valley . . . 565

Anthocyanin pigmentation in Asiatic cottons, genes affecting (Hutchinson, J. B. and Ghose, R.L.M.) . . . 873

Anthracnose disease of sann-hemp (Mitra, M.) . . . 443

Applied entomology, importance of the study of insect ecology in (Ahmad, T.) . . . 156

Asghar, A. G. (McKenzie Taylor, E. and)—Soil deterioration in the canal-irrigated areas of the Punjab . . . 671

Asia and Africa, classification of the cottons of (Hutchinson, J. B. and Ghose, R.L.M.) . . . 233

Asiatic cottons, genes affecting anthocyanin pigmentation in (Hutchinson, J. B. and Ghose, R.L.M.) . . . 873

Atmospheric humidity, germination of fungal spores in relation to (Chowdhury, S.) . . . 653

PAGE

Awnedness and its inheritance in rice (SETHI, R. L. *et al.*) . . . 589

AZIZ, M. A. (BOSE, R. D. and)—Studies in Indian barleys . . . 48

AZMAT SINGH (RAMJI NARAIN and)—Sampling of sugarcane for chemical analysis 601

Azolla pinnata, effect of different cations on the living protoplasm of the root hair of (SEN, B.) 479

B

Bacillus cereus, dissociation of (JOSHI, N. V. and DUTT, S. C.) . . 763

Bamboo-sugarcane hybrids (VENKATRAMAN, T. S.) 513

Bambusa arundinacea (see ' bamboo ') 513

BANERJI, I. and DAS, M. C.—" The Development of Microspores in *Trichosanthes dioica* Roxb." 497

Barleys (Indian), inheritance of some characters responsible for lodging and ear-head characters in an interspecific cross between two Pusa barleys (BOSE, R. D. *et al.*) 48

Base-exchange reactions, equilibrium between Ca and Na ions (MCKENZIE TAYLOR, E. *et al.*) 671

BATHAM, H. N. ; SETHI, R. L. and NIGAM, L. S.—Molasses as Manure in the United Provinces 291

Berhampur Rice Research Station, a few experimental observations in (NARASINGA RAO, M.B.V.) 733

BHALLA, H. R. (AFZAL HUSAIN, M. and)—The bird enemies of the Cotton Leaf-roller at Khanewal (Punjab) 785

BHARDWAJ, N. K. (RAHMAN, K. A. and)—The grape-vine thrips . . 633

BHATIA, H. L. (PRUTHI, H. S. and)—A new Cecidomyid pest of linseed in India 797

BHATIA, K. L. (CHAUDHURI, H. and)—Diseases of tea bush in the Kangra valley 565

BHATNAGAR, M. P. (BOSE, R. D. and)—Studies in Indian barleys . 48

Bihar (North), entomological investigations on the leaf-curl disease of tobacco in (PRUTHI, H. S. and SAMUEL, C. K.) 659

Biology and physiology of *Fusarium vasinfectum* (UPPAL, B. N. and KULKARNI, N. T.) 413

Bird enemies of the Cotton Leaf-roller (AFZAL HUSAIN, M. and BHALLA, H. R.) 785

Black beetle, a pest of sugarcane in Myitkyina district (GHOSH, C. C.) . 907

Blood of *Schistocerca gregaria*, histology of (MATHUR, C. B. and SONI, B. N.) 317

Bolls (cotton), variation in the characters of cotton in relation to the position of (SEN, K. R. and AFZAL, M.) 35

PAGE

Borers of sugarcane, mass-production of *Trichogramma* parasites for the control of (SUBRAMANIAM, T. V.) 149

BOSE, R. D.; AZIZ, M. A. and BHATNAGAR, M. P.—"Studies in Indian Barleys, IV. The Inheritance of some Anatomical Characters responsible for lodging and of some Ear-head Characters in an Interspecific Cross between two Pusa Barleys" 48

BOSE, S. S. and MENON, T. V. G.—"Statistical Notes for Agricultural Workers, No. 23. A Statistical Study of the Deterioration of Yields of the Permanent Manurials, Pusa." 205

BOSE, S. S. (MENON, T. V. G. and)—Statistical notes for agricultural workers 193

Botryodiplodia theobromae, (internal root disease) of tea (CHAUDHURI, H. *et al.*) 571

Brassicae (oleiferous), breeding investigations in (ALI MOHAMMAD and SIKKA, SAWAN MAL) 849

Breeding of plants, studies in the technique of (HUTCHINSON, J. B. and PANSE, V. G.) 531

Broadcast and transplanted paddy, comparison in milling of (SETHI, R. L. *et al.*) 713

Brown-blight disease of tea (CHAUDHURI, H. *et al.*) 569

Brown-spot disease of tea (CHAUDHURI, H. *et al.*) 568

Bruchidae (pulse beetles) of Burma (GHOSH, C. C.) 395

Bruchus albocallosus, life-history of (GHOSH, C. C.) 402

Bruchus analis, life-history of (GHOSH, C. C.) 400

Bruchus chinensis, life-history of (GHOSH, C. C.) 399

Bruchus phaseoli, life-history of (GHOSH, C. C.) 401

Bunt (stinking smut) of wheat in India (MITRA, M.) 459

Burma (Northern), Black beetle pest of sugarcane in (GHOSH, C. C.) . 907

Burma, Phyllody of sesamum in (RHIND, D. *et al.*) 823

Burma, the pulse beetles (Bruchidae) of (GHOSH, C. C.) 395

C

Calcium and sodium ions, equilibrium between, in base-exchange reactions (MCKENZIE TAYLOR, E. *et al.*) 671

Canal-irrigated areas, soil deterioration in (MCKENZIE TAYLOR, E. *et al.*) 671

Castor plant, cyto-genetics of (RICHHARIA, R. H.) 707

Cecidomyid pest of linseed in India (PRUTHI, H. S. and BHATIA, H. L.) 797

Central India, composition of the cotton crops of (HUTCHINSON, J. B. and GHOSE, R. L. M.) 1

Cephaleuros mycoidea (Red rust) of tea (CHAUDHURI, H. *et al.*) . . 572

PAGE

Chakravarty, S. C. (Mehta, P. R. and)—A new disease of *Eleusine coracana* 793

Chaudhuri, H.; Kapur, V. S.; Bhatia, K. L. and Anand, J. S.—" Diseases of the Tea Bush in the Kangra Valley, Punjab, I " . . 565

Cheema, G. S.; Karmarkar, D. V. and Joshi B. M.—" The Cold Storage of Nagpur Oranges (*Citrus aurantium*) " 168

Chemical analysis, sampling of sugarcane for (Ramji Narain and Azmat Singh) 601

Chemical and physical factors, effect of, on sclerotia formation in cotton root-rot (Vasudeva, R. S.) 259

Chemistry and physiology of tropical fruits, ontogenetic drifts in (Singh, B. N. *et al.*) 176

Chowdhury, S.—" Germination of Fungal Spores in relation to Atmospheric Humidity " 653

Citrus aurantium, cold storage of (Cheema, G. S. *et al.*) . . . 168

Classification of the cottons of Asia and Africa (Hutchinson, J. B. and Ghose, R. L. M.) 233

Cold storage of Nagpur oranges (Cheema, G. S. *et al.*) . . . 168

Colletotrichum camelliae (Brown-blight disease of tea) (Chaudhuri, H. *et al.*) 569

Colletotrichum curvatum, causal organism of the anthracnose of sannhemp (Mitra, M.) 443

Copper-blight disease of tea (Chaudhuri, H. *et al.*) 570

Cotton, genetical study of roots in relation to disease-resistance in (Panse, V. G. and Patel, A. F.) 451

——— crops of Central India and Rajputana, composition of (Hutchinson, J. B. and Ghose, R. L. M.) 1

———, effect of certain factors influencing the incidence of root-rot disease of, in the Punjab (Vasudeva, R. S.) 575

———, effect of farmyard manure on fibre characters of (Nayak, H. R.) 877

——— Leaf-roller, bird enemies of (Afzal Husain, M. and Bhalla, H. R.) 785

———, length regularity and percentage of short fibres in . . . 935

———, motes in (Afzal, M.) 487

———, root-rot disease of (Vasudeva, R. S.) 259

———, variation in the characters of, in relation to the position of bolls (Sen, K. R. and Afzal, M.) 35

Cottons (Asiatic), genes affecting anthocyanin pigmentation in (Hutchinson, J. B. and Ghose, R. L. M.) 873

——— of Asia and Africa, classification of (Hutchinson, J. B. and Ghose, R. L. M.) 233

PAGE

Crop ecology, studies in (HUTCHINSON, J. B. and GHOSE, R. L. M.) . 1

Crotalaria juncea, analysis of, with special reference to its use in green-manuring and fibre production (SINGH, B. N. and SINGH, S. N.) . 214

———, an anthracnose disease of (MITRA, M.) . . . 443

———, studies in *Fusarium* wilt of (UPPAL, B. N. and KULKARNI, N. T.) 413

Cyto-genetics of *Ricinus communis* (RICHHARIA, R. H.) . . . 707

Cytological studies in paddy varieties (SETHI, B. L.) 687

D

DAS, M. C. (BANERJI, I. and)—Development of microspores in *Trichosanthes dioica* 497

Dasyneura lini, a new Cecidomyid pest of linseed in India (PRUTHI, H. S. and BHATIA, H. L.) 797

DESAI, S. V. and FAZAL-UD-DIN—"The Influence of Non-nitrifying Organisms on Nitrification". 895

DESHPANDE, R. B. and MALLIK, A. K.—"Studies in Indian Oil-seeds, VI. Some Correlations between Oil-content and other Characters in the Pusa Linseed Hybrids" 841

Desert locust, histology of the blood of (MATHUR, C. B. and SONI, B. N.) 317

Desi (Punjab) cottons, motes in (AFZAL, M.) 487

Disease-resistance in cotton, a genetical study of roots in relation to (PANSE, V. G. and PATEL, A. F.) 451

Dispersion coefficient and degree of alkalisation in deteriorated soil, relation between (MCKENZIE TAYLOR, E. *et al.*) 671

Drifts, ontogenetic, in the physiology and chemistry of tropical fruits (SINGH, B. N. *et al.*) 176

DUTT, S. C. (JOSHI, N. V. and) Dissociation of *Bacillus cereus* . . 763

E

Ear-head characters in an interspecific cross between two Pusa barleys (BOSE R. D. *et al.*) 71

Ecology of crop (HUTCHINSON, J. B. and GHOSE, R. L. M.) . . 1

——— insect, importance of, in applied entomology (AHMAD, T.) 156

Eleusine coracana, a new disease of (MEHTA, P. R. and CHAKRAVARTY, S. C.) 793

Entomology (applied), importance of the study of insect ecology in (AHMAD, T.) 156

PAGE

F

Farmyard manure, effect of, on fibre characters of cotton (NAYAK, H. R.) 877

FAZAL-UD-DIN (DESAI, S. V. and)—Influence of non-nitrifying organisms on nitrification 895

Fibre characters of cotton, effect of farmyard manure on (NAYAK, H. R.) 877

Fibre production, analysis of *Crotalaria juncea*, with special reference to (SINGH, B. N. and SINGH, S. N.) 214

Fibres (short), percentage of, in cotton 935

Field tests of plant breeding material, the design of (HUTCHINSON, J. B. and PANSE, V. G.) 531

Fruits (tropical), ontogenetic drifts in the physiology and chemistry of (SINGH, B. N. *et al.*) 176

Fungal spores, germination of, in relation to atmospheric humidity (CHOWDHURY, S.) 653

Fusarium vasinfectum, physiology and biology of (UPPAL, B. N. and KULKARNI, N. T.) 413

G

GANGULI, P. M. (MITRA, S. K. and)—Inheritance of inner glume colour in rice 126

Genetical study of roots in relation to disease-resistance in cotton (PANSE, V. G. and PATEL, A. F.) 451

Germination of fungal spores in relation to atmospheric humidity (CHOWDHURY, S.) 653

GHOSE, R. L. M. (HUTCHINSON, J. B. and)—Classification of the cottons of Asia and Africa 233

GHOSE, R. L. M. (HUTCHINSON, J. B. and)—Genes affecting anthocyanin pigmentation in Asiatic cottons 873

——————Studies in crop ecology . . 1

GHOSH, C. C.—“ The Black Beetle (*Alissonotum impressicolle* Arr.), a Pest of Sugarcane in Myitkyina District in Northern Burma ” . 907

——————“ The Pulse Beetles (*Bruchidae*) of Burma ” . . 395

Glume (inner) colour in rice, inheritance of (MITRA, S. K. and GANGULI, P. M.) 126

Gossypium (see ‘ cotton ’) . . 1, 35, 233, 259, 451, 487, 575, 873, 877, 935

Grape-vine thrips (RAHMAN, K. A. and BHARDWAJ, N. K.) . . . 633

Grassland Congress (Fourth International) 349

Green-flowering (see ‘ phyllody ’) 823

Green-manuring, analysis of *Crotalaria juncea* with special reference to its use in (SINGH, B. N. and SINGH, S. N.) 214

PAGE

Grey blight of tea (CHAUDHURI, H. *et al.*) 566

Growth rate of rice, effect of watering on (SEN, P. K.) . . . 89

Guignardia camelliae (Copper blight disease of tea) (CHAUDHURI, H. *et al.*) 570

GUPTA, P. S. (SETHI, R. L. and)—Milling of rice 713

GUPTA, S. S. (SINGH, B. N. and)—Physiology and chemistry of tropical fruits 176

H

Hendersonia theicola (a stem disease of tea) (CHAUDHURI, H. *et al.*) . 572

Heterosis in rice (KADAM, B. S. *et al.*) 118

Hulling properties of different types of rice (SETHI, R. L. *et al.*) . . 713

Humidity, atmospheric, germination of fungal spores in relation to (CHOWDHURY, S.) 653

HUTCHINSON, J. B. and GHOSE, R. L. M.—" A Note on two New Genes affecting Anthocyanin Pigmentation in Asiatic Cottons " . . 873

——————————" Studies in Crop Ecology, I. The Composition of the Cotton Crops of Central India and Rajputana " 1

——————————" The Classification of the Cottons of Asia and Africa " 233

HUTCHINSON, J. B. and PANSE, V. G.—" Studies in Plant Breeding Technique, II. The Design of Field Tests of Plant-breeding Material " 531

Hybrids of Pusa linseed, some correlations between oil-content and other characters of (DESHPANDE, R. B. and MALLIK, A. K.) . . 841

Hybrids of sugarcane and bamboo (VENKATRAMAN, T. S.) . . . 513

Hydrogen-ion changes in the medium and its relation to plant-growth (SINGH, B. N. and MITRA, G. P.) 327

I

India, a new Cecidomyid pest of linseed in (PRUTHI, H. S. and BHATIA, H. L.) 797

——(Northern) types of tobacco leaf-curl in (PAL, B. P. and TANDON R. K.) 363

——, *Pyrilla* pest of sugarcane in (PRUTHI, H. S.) 511

——, stinking smut or bunt of wheat in (MITRA, M.) 459

Indian barleys, inheritance of some anatomical characters responsible for lodging, and of ear-head characters in an interspecific cross between two Pusa barleys (BOSE, R. D. *et al.*) 48

—— oil-seeds, studies in (DESHPANDE, R. B. and MALLIK, A. K.) . 841

PAGE

Inheritance of awnedness in rice (SETHI, R. L. *et al.*) 589

————of inner glume colour in rice (MITRA, S. K. and GANGULI, P. M.) 126

————of sheathed ear in rice (SETHI, R. L. *et al.*) . . . 134

————of some anatomical characters responsible for lodging (BOSE, R. D. *et al.*) 50

Insect ecology, importance of, in applied entomology (AHMAD, T.) . 156

Internal root disease of tea (CHAUDHURI, H. *et al.*) 571

International Grassland Congress (Fourth) 349

Interspecific cross between two Pusa barleys, ear-head characters in (BOSE, R. D. *et al.*) 489

Irrigated (canal) areas, soil deterioration in (MCKENZIE TAYLOR, E. *et al.*) 671

J

JOSHI, B. M. (CHEEMA, G. S. and)—Cold storage of Nagpur oranges . 168

JOSHI, N. V. and DUTT, S. C.—" Studies on the Dissociation of *Bacillus cereus*, an Organism associated with Plants affected with Mosaic Disease " 763

K

Kachcha, *sela* and parboiled rices, milling of (SETHI, R. L. *et al.*) . . 713

KADAM, B. S.; PATIL, G. G. and PATANKAR, V. K.—" Heterosis in Rice " 118

Kangra valley, diseases of tea bush in (CHAUDHURI, H. *et al.*) . . 565

KAPUR, V. S. (CHAUDHURI, H. and)—Diseases of tea bush in the Kangra Valley 565

KARMARKAR, D. V. (CHEEMA, G. S. and)—Cold storage of Nagpur oranges 168

Khanewal, bird enemies of the Cotton Leaf-roller at (AFZAL HUSAIN, M. and BHALLA, H. R.) 785

KULKARNI, N. T. (UPPAL, B. N. and)—Studies in *Fusarium* wilt of sann-hemp 413

L

LAL, T. B.—" *Uromyces rumicis* (Schum.) Winter: a new record for India " 933

Late-blight, relative susceptibility of some potato varieties to (MUNDKUR, B. B. *et al.*) 627

Leaf-curl disease of tobacco, entomological investigations on (PRUTHI, H. S. and SAMUEL C. K.) 659

PAGE

Leaf-curl of tobacco in Northern India (PAL, B. P. and TANDON, R. K.) 363

Leaf-roller of cotton, bird enemies of (AFZAL HUSAIN, M. and BHALLA, H. R.) 785

Linseed, a new Cecidomyid pest of (PRUTHI, H. S. and BHATIA, H. L.) 797

——- hybrids, some correlations between oil-content and other characters in (DESHPANDE, R. B. and MALLIK, A. K.) 841

Linum (see 'linseed') 797, 841

Locust (Desert), histology of the blood of (MATHUR, C. B. and SONI, B. N.) 317

Lodging in barleys, inheritance of some anatomical characters responsible for (BOSE, R. D. *et al.*) 50

LUTHRA, JAI CHAND ; ABDUS SATTAR and ABDUL GHANI, M.—"A Comparative Study of Species of Septoria occurring on Wheat" . . 271

M

MALLIK, A. K. (DESHPANDE, R. B. and)—Studies in Indian oil-seeds. 841

Manures and manuring—

Analysis of *Crotalaria juncea* with special reference to its use in green manuring and fibre production (SINGH, B. N. and SINGH, S. N.) 214

Effect of farmyard manure on fibre characters of cotton (NAYAK, H. R.) 877

Molasses as manure in the United Provinces (BATHAM, H. N. *et al.*) . 291

Statistical study of the yields of the permanent manurials, Pusa (MENON, T. V. G. and BOSE, S. S.) 193

Statistical study of the deterioration of yields of the permanent manurials (BOSE, S. S. and MENON T. V. G.) 205

MATHUR, C. B. and SONI, B. N.—"Studies on *Schistocerca gregaria* Forsk., IX. Some Observations on the Histology of the Blood of the Desert Locust" 317

McKENZIE TAYLOR, E.; PURI, AMAR NATH and ASGHAR, A. G.—"Soil Deterioration in the Canal-irrigated Areas of the Punjab." (Abstract) 671

MEHTA, P. R. and CHAKRAVARTY, S. C.—"A New Disease of *Eleusine coracana* Gaertn.)" 793

MEHTA, T. R. (SETHI, R. L. and)—Awnedness and its inheritance in rice 589

——————————Inheritance of sheathed ear in rice 134

——————————Milling of rice 713

MENON, T. V. G. and BOSE, S. S.—"Statistical Notes for Agricultural Workers, No. 22. A Statistical Study of the Yields of Permanent Manurials, Pusa." 193

PAGE

MENON, T. V. G. (BOSE, S. S. and)—Statistical notes for agricultural workers, No. 23 205

Meteorology (agricultural) (NARASIMHAN, M. and RAMDAS, L. A.) . 745

Microspores, development of, in *Trichosanthes dioica* (BANERJI, I. and DAS, M. C.) 497

Milling of rice in the United Provinces (SETHI, R. L. *et al.*) . . . 713

MITRA, G. P. (SINGH, B. N. and)—Plant-growth in relation to hydrogen-ion changes in its medium 327

MITRA, M.—" An Anthracnose Disease of Sann-hemp " . . . 443

——— " Studies on the Stinking Smut or Bunt of Wheat in India " . 459

MITRA, S. K. and GANGULI, P. M.—" Inheritance of Inner Glume Colour in Rice " 126

Molasses as manure in the United Provinces (BATHAM, H. N. *et al.*) . 291

Mosaic disease, *Bacillus cereus*—an organism associated with plants affected with (JOSHI, N. V. and DUTT, S. C.) 763

Motes in cotton (AFZAL, M.) 487

MUNDKUR, B. B.; PAL, B. P. and PUSHKAR NATH—" Relative Susceptibility of some Wild and Cultivated Potato Varieties to an Epidemic of Late-blight at Simla in 1936 " 627

Myitkyina district, Black beetle pest of sugarcane in (GHOSH, C. C.) . 907

Mysore, mass-production of *Trichogramma* parasites for control against sugarcane borers in (SUBRAMANIAM, T. V.) 149

N

Nagpur oranges, the cold storage of (CHEEMA, G. S. *et al.*) . . . 168

NARASIMHAN, M. and RAMDAS, L. A.—" Agricultural Meteorology : the Prediction of the Minimum Temperature on Clear Days at Selected Stations in India " 745

NARASINGA RAO, M. B. V.—" A Note on a few Experimental Observations in the Rice Research Station, Berhampur " 733

NAYAK, H. R.—" Effect of Farmyard Manure on Fibre Characters of Cotton " 877

Nicotiana (see ' tobacco ') 363, 659

NIGAM, L. S. (BATHAM, H. N. and)—Molasses as manure in the United Provinces 291

Nitrification, influence of non-nitrifying organisms on (DESAI, S. V. and FAZAL-UD-DIN) 895

Northern India, types of tobacco leaf-curl in (PAL, B. P. and TANDON, R. K.) 363

PAGE

O

ODELL, F. D. (RHIND, D. and)—Phyllody of sesamum in Burma . 823

Oil-content and other characters in the Pusa linseed hybrids, some correlations between (DESHPANDE, R. B. and MALLIK, A. K.) . 841

Oil-seeds (Indian), studies in (DESHPANDE, R. B. and MALLIK, A. K.) . 841

Ontogenetic drifts in the physiology and chemistry of tropical fruits (SINGH, B. N. *et al.*) 176

Oranges (Nagpur), the cold storage of (CHEEMA, G. S. *et al.*) . . 168

Oryza sativa (see 'rice') . . . 89, 118, 126, 134, 589, 687, 713, 738, 863

P

Paddy (see 'rice') 89, 118, 126, 134, 589, 687, 713, 738, 863

PAL, B. P. and TANDON, R. K.—" Types of Tobacco Leaf-curl in Northern India " 363

————(MUNDKUR, B. B. and)—Relative susceptibility of wild and cultivated potato varieties to late-blight 627

PANSE, V. G. (HUTCHINSON, J. B. and)—Design of field tests of plant-breeding material 531

PANSE, V. G. and PATEL, A. F.—" A Genetical Study of Roots in relation to Disease-resistance in Cotton " 451

Parboiled, *sela* and *kachcha* rices, milling of (SETHI, R. L. *et al.*) . . 713

PATANKAR, V. K. (KADAM, B. S. and)—Heterosis in rice . . . 118

PATEL, A. F. (PANSE, V. G. and)—A genetical study of roots in relation to disease-resistance in cotton 451

PATIL, G. G. (KADAM, B. S. and)—Heterosis in rice 118

Permanent manurials, a statistical study of the yields of (MENON, T. V. G. and BOSE, S. S.) 193

————————deterioration of yields of the (BOSE, S. S. and MENON, T. V. G.) 205

Pestalozzia theae (Grey blight disease of tea) (CHAUDHURI, H. *et al.*) . 566

Petalody (see 'phyllody') 823

Phoma theicola (Brown spot disease of tea) (CHAUDHURI, H. *et al.*) . 568

Phyllody of sesamum in Burma (RHIND, D. *et al.*) 823

Physical and chemical factors, effect of, on sclerotia formation in cotton root-rot (VASUDEVA, R. S.) 259

Physiology and biology of *Fusarium vasinfectum* (UPPAL, B. N. and KULKARNI, N. T.) 413

Physiology and chemistry of tropical fruits, ontogenetic drifts in (SINGH, B. N. *et al.*) 176

Phytophthora infestans (late-blight), relative susceptibility of some potato varieties to (MUNDKUR, B. B. *et al.*) 627

PAGE

Pigmentation (anthocyanin) in Asiatic cottons, genes affecting (Hutchinson, J. B. and Ghose, R. L. M.) 873

Plant-breeding material, the design of field tests of (Hutchinson, J. B. and Panse, V. G.) 531

Plant-growth in relation to hydrogen-ion changes in its medium (Singh, B. N. and Mitra, G. P.) 327

Plot-size, experimental observations on, in the Rice Research Station, Berhampur (Narasinga Rao, M. B. V.) 733

Potato varieties (wild and cultivated), relative susceptibility of, to late-blight (Mundkur, B. B. *et al.*) 627

Prediction of the minimum temperature on clear days at selected stations in India (Narasimhan, M. and Ramdas, L. A.) . . . 745

Protoplasm (living) of the root hair of *Azolla pinnata*, effect of different cations on (Sen, B.) 479

Pruthi, Hem Singh—"On the *Pyrilla* Pest of Sugarcane in India" . 511

——— and Bhatia, H. L.—"A New Cecidomyid Pest of Linseed in India" 797

——— and Samuel, C. K.—"Entomological Investigations on the Leaf-curl Disease of Tobacco in North Bihar" . . 659

Pulse beetles (Bruchidae) of Burma (Ghosh, C. C.) 395

Punjab, bird enemies of the Cotton Leaf-roller in the (Afzal Husain, M. and Bhalla, H. R.) 785

———, breeding investigations in some of the oleiferous *Brassicæ* of the (Ali Mohammad and Sikka, Sawan Mal) . . . 849

——— *desi* cottons, motes in (Afzal, M.) 487

——— (Kangra valley), diseases of tea bush in (Chaudhuri, H. *et al.*) 565

———, root-rot disease of cotton in the (Vasudeva, R. S.) . . 259, 575

Puri, A. N. (McKenzie Taylor, E. and)—Soil deterioration in the canal-irrigated areas of the Punjab 671

Pusa, statistical study of the yields of permanent manurials at (Menon, T. V. G. and Bose, S. S.) 193

———, statistical study of the deterioration of yields of the permanent manurials at (Bose, S. S. and Menon, T. V. G.) 205

Pusa barleys, inheritance of some anatomical characters responsible for lodging and of some ear-head characters in an interspecific cross between (Bose, R. D. *et al.*) 48

——— linseed hybrids, some correlations between oil-content and other characters in (Deshpande, R. B. and Mallik, A. K.) 841

Pushkar Nath (Mundkur, B. B. and)—Relative susceptibility of wild and cultivated potato varieties to late-blight 627

Pyrilla pest of sugarcane in India (Pruthi, H. S.) 511

PAGE

R

Ragi (*Eleusine coracana*), a new disease of (MEHTA, P. R. and CHAKRAVARTY, S. C.) 793

RAHMAN, KHAN A. and BHARDWAJ, NAND KISHORE—" The Grape-vine Thrips (*Rhipiphorothrips cruentatus* Hood) (Thripidae : Terebrantia : Thysanoptera) " 633

RAJASEKHARA MUDALIAR, C. (RAMIAH, K. and).—Colours in the rice grain 863

Rajputana, composition of the cotton crops of (HUTCHINSON J. B. and GHOSE, R. L. M.) 1

RAMDAS, L. A. (NARASIMHAN, M. and)—Agricultural meteorology . 745

RAMIAH, K. and RAJASEKHARA MUDALIAR, C.—" Colours in the Rice Grain " 863

RAMJI NARAIN and AZMAT SINGH—" Sampling of Sugarcane for Chemical Analysis, I." 601

Red rust of tea (CHAUDHURI, H. *et al.*) 572

Reviews and book-notices—

" The Experimental Production of Haploids and Polyploids " . 351

" The South American Potatoes and their Breeding Value " . . 351

" An Outline of Cytological Technique for Plant Breeders " . . 515

" Vegetative Propagation of Tropical and Sub-tropical Fruits ", by G. ST. CL. FIELDEN and R. J. GARNER 517

" Horticultural Aspects of Woolly Aphis Control together with a Survey of Literature" by R. M. GREENSLADE . . . 518

" Een Bijdrage Tot DeKennis Omtrent Het Bloeien En Het Vruchtdragende Vermogen Van Den Mangga *Mangifera indica* L.) " (A Contribution to the Knowledge of the Flowering and Fruiting Habits of the Mango Tree, *Mangifera indica* L.) by A. P. C. BIJHOUWER 519

" First Guide to Agricultural Literature " 673

" Proceedings of the Association of Applied Biologist, October 1936 " 937

" Drilling Mud : its Manufacture and Testing ", by P. EVANS and A. REID 809

" Annual Review of Biochemical and Allied Research in India, Vol. VII, 1936 " 810

" The Plant Diseases of Great Britain ", by G. C. AINSWORTH . . 810

" Herbage Abstracts, Vol. 7, No. 1, March 1937 " 810

RHIND, D. ; ODELL, F. D. and THET SU, U—" Observations on Phyllody of Sesamum in Burma " 823

Rhipiphorothrips cruentatus (grape-vine thrips) (RAHMAN, K. A. and BHARDWAJ, N. K.) 633

PAGE

Rice, awnedness and its inheritance in (SETHI, R. L. *et al.*) 589
———, correlation of some characters on yield in (NARASINGA RAO, M. B. V.) 738
———grain, colours in (RAMIAH, K. and RAJASEKHARA MUDALIAR, C.) 863
———, heterosis in (KADAM, B. S. *et al.*) 118
———, inheritance of inner glume colour in (MITRA, S. K and GANGULI, P. M.) 126
———, inheritance of sheathed ear in (SETHI, R. L. *et al.*) . . . 134
———, milling of, in the United Provinces, (SETHI, R. L. *et al.*) . . 713
Rice Research Station, Berhampur, a few experimental observations in (NARASINGA RAO, M. B. V.) 733
Rice, studies in the water relations of (SEN, P. K.) 89
———, varieties, cytological studies in (SETHI, B. L.) 687
RICHHARIA, R. H. " A Note on the Cyto-genetics of *Ricinus communis* L." 707
Ricinus communis, cyto-genetics of (RICHHARIA, R. H.) 707
Root-rot disease of cotton in the Punjab (VASUDEVA, R. S.) . . 259, 575

S

Saccharum (see ' sugarcane ') 511, 513, 601, 907
Sampling of sugarcane for chemical analysis (RAMJI NARAIN and AZMAT SINGH) 601
Sann-hemp (*Crotalaria juncea*), analysis of, with special reference to its use in green-manuring and fibre production (SINGH, B. N. and SINGH, S. N.) 214
Sann-hemp, studies in *Fusarium* wilt of (Uppal, B. N. and KULKARNI, N. T.) 413
Sann-hemp, an anthracnose disease of (MITRA, M.) . . . 443
SAMUEL, C. K. (PRUTHI, H. S. and)—Leaf-curl disease of tobacco . 659
Scabbing of leaves in tea (CHAUDHURI, H. *et al.*) 570
Schistocerca gregaria, histology of the blood of (MATHUR, C. B. and SONI, B. N.) 317
Sclerotia formation in cotton root-rot, effect of physical and chemical factors on (VASUDEVA, R. S.) 259
Sela, parboiled and *kachcha* rices, milling of (SETHI, R. L. *et al.*) . 713
SEN, B.—" The Effect of different Cations on the Living Protoplasm of the Root Hair of *Azolla pinnata* " 479
SEN, K. R. and Afzal, Mohammad—" Variation in the Characters of Cotton in relation to the Position of Bolls on the Plant." . . 35

PAGE

SEN P. K.—" Studies in the Water Relations of Rice, I. Effect of Watering on the Rate of Growth and Yield of four Varieties of Rice " 89

Septoria species occurring on wheat (LUTHRA, J. C. *et al.*) . . . 271

SETHI, B. L.—" Cytological Studies in Paddy Varieties " . . . 687

SETHI, B. L. (SETHI, R. L. and)—Awnedness and its inheritance in rice 589

— ————————Inheritance of sheathed ear in rice 134

————————Milling of rice 713

SETHI, R. L. (BATHAM, H. N. and)—Molasses as manure in the United Provinces 291

SETHI, R. L. ; SETHI, B. L. and MEHTA, T. R.—" Awnedness and its Inheritance in Rice " 589

————————" Inheritance of Sheathed Ear in Rice " 134

————————and Gupta P. S.—"Observations on the Milling of Rice in the United Provinces " . . 713

Sesamum orientale, phyllody of, in Burma (RHIND, D. *et al.*) . . 823

Sesamum, phyllody of, in Burma (RHIND, D. *et al.*) 823

SESHAGIRI, P. V. V. (SINGH, B. N. and)—Ontogenetic drifts in the physiology and chemistry of tropical fruits 176

Sheathed ear in rice, inheritance of (SETHI, R. L. *et al.*) . . . 134

SIKKA, SAWAN MAL (ALI MOHAMMAD and)—Breeding investigations in oleiferous *Brassicæ* of the Punjab 849

Simla, relative susceptibility of some potato varieties to late-blight at (MUNDKUR, B. B. *et al.*) 627

SINGH, B. N. and MITRA, G. P.—" Plant-growth in relation to Hydrogen-ion Changes in its Medium " 327

SINGH, B. N. and SINGH, S. N.—" Analysis of *Crotalaria juncea* with special reference to its Use in Green Manuring and Fibre Production " (Abstract) 214

————————; SESHAGIRI, P. V. V. and GUPTA, S. S.—" Ontogenetic Drifts in the Physiology and Chemistry of Tropical Fruits under Orchard Conditions " 176

SINGH, S. N. (SINGH, B. N. and)—Analysis of *Crotalaria juncea* . . 214

Smut (stinking) or bunt of wheat in India (MITRA, M.) . . . 459

Sodium and calcium ions, equilibrium between, in base-exchange reactions (MCKENZIE TAYLOR, E. *et al.*) 671

Soil deterioration in the canal-irrigated area (MCKENZIE TAYLOR, E. *et al.*) 671

Solanum (see ' potato ') 627

PAGE

SONI, B. N. (MATHUR, C. B. and)—Histology of the blood of the Desert Locust 317

Spores (fungal), germination of, in relation to atmospheric humidity (CHOWDHURY, S.) 653

Statistical notes for agricultural workers (MENON, T. V. G. and BOSE, S. S.) 193

———————(BOSE, S. S. and MENON, T. V. G.) 205

Stem-disease of tea (CHAUDHURI, H. *et al.*) 572

Stinking smut or bunt of wheat in India (MITRA, M.) 459

Storage (cold) of Nagpur oranges (CHEEMA, G. S. *et al.*) . . . 168

SUBRAMANIAM, T. V.—" Preliminary Experiments on the Mass-production of *Trichogramma* Parasites for Control against Sugarcane Borers in Mysore " 149

Sugarcane-bamboo hybrids (VENKATRAMAN, T. S.) 513

————, Black beetle pest of (GHOSH, C. C.) 907

————borers, mass-production of *Trichogramma* parasites for control against (SUBRAMANIAM, T. V.) 149

————, *Pyrilla* pest of (PRUTHI, H. S.) 511

————, sampling of, for chemical analysis (RAMJI NARAIN and AZMAT SINGH) 601

Sylepta derogata (Cotton Leaf-roller), the bird enemies of (AFZAL HUSAIN, M. and BHALLA, H. R.) 785

T

TANDON, R. K. (PAL, B. P. and)—Tobacco leaf-curl in Northern India 363

Tea bush, diseases of, in the Kangra Valley (CHAUDHURI, H. *et al.*) . 565

Temperature (minimum), prediction of, on clear days (NARASIMHAN, M. and RAMDAS, L. A.) 745

THET SU, U (RHIND, D. and)—Phyllody of sesamum in Burma . . 823

Thrips of grape-vine (RAHMAN, K. A. and BHARDWAJ, N. K.) . . 633

Tilletia indica (bunt) of wheat in India (MITRA, M.) 459

Tobacco, entomological investigations on the leaf-curl disease of (PRUTHI, H. S. and SAMUEL, C. K.) 659

————, leaf-curl in Northern India (PAL, B. P. and TANDON, R. K.) 363

Transplanted and broadcast paddy, comparison in milling of (SETHI, R. L. *et al.*) 713

Trichogramma parasites for control against sugarcane borers, mass-production of (SUBRAMANIAM, T. V.) 149

Trichosanthes dioica, development of microspores in (BANERJI, I. and DAS, M. C.) 497

Tropical fruits, ontogenetic drifts in the physiology and chemistry of (SINGH, B. N. *et al.*) 176

Triticum (see ' wheat ') 271, 459

PAGE

U

United Provinces, milling of rice in the (SETHI, R. L. *et al.*) . . 713
——————, molasses as manure in the (BATHAM, H. N. *et al.*) 291
UPPAL, B. N. and KULKARNI, N. T.—" Studies in *Fusarium* Wilt of Sann-hemp, I. The Physiology and Biology of *Fusarium vasinfectum* Atk." 413
Uromyces rumicis, a new record for India (LAL, T. B.) . . . 933

V

VASUDEVA, R. SAHAI.—" Studies on the Root-rot Disease of Cotton in the Punjab, III. The Effect of some Physical and Chemical Factors on Sclerotia Formation " 259
——————IV.—" The Effect of certain Factors influencing Incidence of the Disease " 575
VENKATRAMAN, T. S.—" Sugarcane-bamboo Hybrids " . . . 513

W

Water-relations of rice, studies in (SEN, (P. K.) 89
Wheat, species of *Septoria* occurring on (LUTHRA, J. C. *et al.*) . . 271
Wheat, stinking smut or bunt of (MITRA, M.) 459

Y

Yield of rice, effect of watering on (SEN, P. K.) 89
——————, correlation of some characters on (NARASINGA RAO, M. B. V.) 738
Yields of the permanent manurials, a statistical study of (MENON, T. V. G. and BOSE, S. S.) 193
Yields of the permanent manurials, a statistical study of the deterioration of (BOSE, S. S. and MENON, T. V. G.) 205

GIPD—38—311 IC of AR—8-10-38—800.

ORIGINAL ARTICLES

STUDIES IN CROP ECOLOGY

I. THE COMPOSITION OF THE COTTON CROPS OF CENTRAL INDIA AND RAJPUTANA

BY

J. B. HUTCHINSON

AND

R. L. M. GHOSE

Institute of Plant Industry, Indore, Central India

(Received for publication on 14th October 1936)

(With one text-figure)

CONTENTS

PAGE
I. INTRODUCTION 1
II. HISTORY 3
III. CENSUS 7
IV. CROP ANALYSIS 16
V. DISCUSSION 24
VI. SUMMARY 32
VII. ACKNOWLEDGMENTS 34
REFERENCES 34

I. INTRODUCTION

THE study here reported developed from an attempt to secure from the crop itself satisfactory evidence concerning the deterioration which is alleged to have taken place in Malvi cotton in recent years. The current beliefs concerning this deterioration can best be indicated by summarising the views expressed by the late Mr. Bernard Coventry [1916] in a Note on a Tour in Central India, and in evidence before the Indian Cotton Committee [Coventry, 1920], and by quotation from Howard & Howard [1929].

Coventry [1916]: " In former times Malvi cotton was in great request and carried a very high premium, but of recent years the quality has become considerably deteriorated. We discovered this to be due to mixing with inferior types ". In his evidence before the Indian Cotton Committee [1920], after describing what he considered to be the essential characteristics of the fine Malvi and coarse Nimari cottons, he stated that the two types were very commonly grown together, and further mixing took place in ginneries. He added that it was very difficult to find either pure Malvi or pure Nimari cottons, but that in regions remote from railways pure crops still existed. He attributed the mixing to the excellent transport facilities of modern times, which tended to encourage the import of inferior cottons into superior tracts, and to the greater premium given by ginners for high ginning per cent than for good staple.

Howard & Howard [1929] stated, " As is well known, the Malwa plateau produced in the past a very good type of cotton, soft in texture and of moderate length ; of recent years a great deterioration has taken place owing to the influx of the *roseum* ".

While deterioration was recognised in Nimari cotton, nothing like the same importance appears to have been attached to it as to the supposed deterioration in Malvi.

A study of Central Indian conditions reveals two serious flaws in these statements. The first is that no other crop at present grown in Malwa is pure, and all the available evidence goes to show that the primitive condition of agricultural crops is one of heterogeneity rather than of uniformity. The second is that communications through the narrow Vindhyan range which separates Malwa from Nimar have been good enough to allow of interchange of seed for many hundreds of years.

The theory of invasion by *Roseum* types of cotton from Nimar is easily tested by an examination of a large range of samples from cultivators' fields, and as it appeared that no data had been collected, it was decided to carry out as extensive an examination of the crop as possible in 1933-34.

As we have elsewhere [Hutchinson & Ghose (In press)] dispensed with the commonly accepted nomenclature of the sub-types of cultivated Asiatic cottons, it will be as well to define the meaning we attach to the names used in what follows :

Only two species of *Gossypium* are found in Central India :—

(1) *G. arboreum* L. var. *neglectum*, Watt forma *bengalensis* (H. & G.), the indigenous, or *desi* type.

(2) *G. hirsutum* L., the introduced Upland type.

The commonly occurring forms of *G. arboreum* var. *neglectum* forma *bengalensis* differ in respect of simple Mendelian factors for corolla colour [Hutchinson, 1931] and leaf-shape [Hutchinson 1934], giving four types :—

Corolla colour	*Leaf-shape*	*Name*
Yellow	Narrow	*Verum*
Yellow	Broad	*Malvense*
White	Narrow	*Roseum*
White	Broad	*Cutchicum*

These names will be used for convenience, but it should be understood that nothing more is implied than a particular constitution with regard to corolla colour and leaf-shape. Two distinct types of *G. hirsutum* L. (Upland) are met with. The Nimar type is known as *Malan* and appears to be a typical acclimatised form of Upland Georgian. The Malwa type is distinct, and shows definite evidence of the effect of later introductions of Cambodia. The first recorded distribution was that of Upland Georgian made in both Malwa and Nimar by Sir C. M. Wade in 1842 [Return (1847) p. 200]. More recently, the late Mr. Bernard Coventry introduced Cambodia into Malwa from Madras in considerable quantity. The resulting type is known as Cambodia, and in certain small areas where it is grown pure, it is probably still of predominantly Cambodia descent, but as no critical evidence is obtainable as to the proportion in which Cambodia and Georgian have contributed to the ancestry of the Upland component of the mixed Malvi crop, we propose to refer to it simply as Malwa Upland.

II. History

Early references to the cottons of Central India are rather scattered, but it has been possible to obtain information of considerable value from the fact that the fine muslin industry of Chanderi drew its raw material very largely from the Indore market. The most valuable source of information which we have been able to find concerning the cottons formerly grown in India is the Return: Cotton (India) [1847]. From it information has been obtained on the cottons grown in Central India at that time, which may be classified under two heads: (1) The ordinary cotton of Malwa was valued above the inferior cotton of Bundelcund, but not as highly as that of Berar (Amraotee) or of Nimar. (2) A particularly fine cotton, used exclusively in Chanderi for spinning fine yarn for the manufacture of fine muslins, was grown in a small area near Mandeleshwar on the Nerbudda river. The following quotations are given in support of these conclusions:—

S. Fraser, Esq., Agent to the Lieutenant-Governor in Bundelcund, [Return (1847) No. 78]: "The Berar or Amraotee cotton is longer, finer, and softer than the Bundelcund; but this, I am told, is owing to some peculiarity of soil and atmosphere; it is not, I believe, a peculiar class of plant; the produce sells for double the price of Bundelcund.

"In Malwa, generally, the cotton is the same as at Bundelcund, but is grown in a different soil. In Bundelcund the richest soils are selected, but in Malwa what we in Bundelcund call 'Rankar', a coarse, gravelly soil. Malwa is, however, a damp, moist climate, and the soil well supplied with water, which may render the inferior soil more fruitful than we find it in Bundelcund." T. J. Finnie, Esq., Cotton Planter, [Return (1847) No. 166]: "Since the report (on a valuation of a series of samples of Malwa cottons sent by Sir C. M. Wade, Political Resident at Indore) was written I have taken a cursory view of Sir John Malcolm's account of the climate and soil of Malwa, and must attribute the general superiority of the cotton of the country to the mildness of the climate, the greater regularity of the rains, and superior fertility of the soil ". In the accompanying remarks on fifty-nine samples of Malwa cotton, Mr. Finnie dealt with the intrinsic quality of the cotton as well as with its market value as affected by trash, leaf, etc. In brief, he found that the samples varied from potentially very superior types, which " could not be distinguished from pretty good New Orleans cotton ", to " very inferior staple ", but in most cases their value was greatly reduced by the presence of large amounts of foreign matter.

The available evidence concerning the exceptional cotton grown in Nimar for the use of the Chanderi hand-spinners is contained in reports by Surgeon R. H. Irvine, Capt. J. Abbott, Mr. T. J. Finnie, the Bombay Chamber of Commerce and a firm of Liverpool brokers.

Surgeon Irvine's report [Return (1847) No. 75] is of importance only from two points of view. (1) He had no great opinion of the quality of the cotton (which he calls *Nurma*). He stated that a " common American cotton " grown by him at Gwalior " is finer and in every respect better than the *Nurma* cotton ; the Chanderee people say so................ ". (2) His report of a single year's trial of this cotton on three *bighas* of land at Heerawal and two *bighas* at Susarde (villages near Chanderi) appears to have given rise to the statement found in later publications [Watt, 1890] that the cultivation of this particular cotton was confined to five *bighas* of land in all, in these two villages. Irvine's report itself, however, is quite clear that the cotton was grown near " Cholai Mahaisoor " on the Nerbudda and thence sent to Chanderi.

Capt. Abbott was stationed at Mandeleshwar on the Nerbudda at the time of the enquiries to which his report [Return (1847) No. 145] was a reply, and he was consequently in a position to give first-hand information. He first distinguished between *Nurma*, which he described as a perennial cotton, no doubt the same type of *G. arboreum* as is now known by that name, and what he calls " *M'hahlie* ", which was an annual, and which provided the fine cotton which was exported to Chanderi for spinning. He said that it " cannot be indigenous in Nimar ", was " harvested about 40 days later than the common kind, *i.e.*, about the end of March ", and " It has never been cleaned in Nimar, but was exported to Chundairee in the pod ". From this it is a fair inference that it was a closed-bolled type of *G. herbaceum* L. or *G. herbaceum* L. var. *frutescens* Delile. Further, " The growth of M'hahlie was confined to two Pergunnahs of Nimar, Dhargaon and Kussrode, and to a few villages of those Pergunnahs ; I should add to a single village of Muhaiswah ". He stated that although there was a flourishing spinning and weaving industry in Nimar, the local artisans preferred the common cotton as it was considered stronger and more suitable for the relatively coarse cloths which they wove. He estimated the yield of *M'hahlie* as from five to six maunds ("of 80 rupees") on unirrigated land, as against four maunds of the common (or *Guandailah*) cotton per *bigha* of 22,500 sq. ft. At the time he wrote, the Chanderi industry had declined to such an extent that *M'hahlie* cotton actually sold in Nimar at such a discount as to counterbalance the advantage of its higher yield, and Capt. Abbott predicted that it would speedily disappear unless some encouragement were given to its cultivation. His prediction has unfortunately been fulfilled, and no trace of it can now be found.

With his report, Capt. Abbott forwarded to Sir C. M. Wade at Indore, seven samples. Of these , three were of *M'hahlie* cotton, one of *Nurma* cotton and one of *Nurma* seed, one of the common (" *Goondaslah* ") cotton of Nimar, and one of the common cotton of Oomrouthee (*Berari*). Of these, Sir C. M. Wade appears to have forwarded six to the Secretary to the Government, North-Western Provinces, Agra [Return (1847) No. 144]. The remaining one (of *M'hahlie*) was probably sent with a despatch referred to in his covering letter (No. 144), to the Honourable the Governor-in-Council, Bombay.

The samples sent to Agra were forwarded, together with Sir C. M. Wade's samples of Malwa cotton, to Mr. T. J. Finnie, Cotton Planter, for his opinion. Concerning the first sample of *M'hahlie* cotton, he stated, " This is a very fine long-stapled cotton, and if it could be procured in sufficient quantities, would be but little inferior to Sea Island ". The second sample he said was a shade shorter and less fine, but was " a first rate article ". The sample of *Nurma* he considered disappointing and " not better than some of the common cotton received from Malwa ", presumably referring to the Malwa cottons, his report on which is summarised above. He considered that the *Goondaslah* cotton of Nimar and the Oomrouthee sample were indistinguishable, and were of very good quality. He said, " In this article I realise my anticipation of the Nurmah cotton ", and " This, as a common cotton, is very good ; indeed not inferior to good ' Uplands ' or New Orleans cotton ".

A sample of *M'hahlie* which we conclude must have been one of Capt. Abbott's, was sent by the Secretary to Government, Bombay, to the Bombay Chamber of Commerce for valuation. Their opinion [Return (1847) No. 429] was " that it is of little value as an article of commerce, the staple being weak and uneven, and exhibiting a knotted appearance ; results which, whether it has been hand-picked or cleaned by the churka, are no doubt owing to the difficulty of separating it from the seed ".

One further report on *M'hahlie* is available. It is the opinion of a firm of Liverpool brokers on two samples which appear to have been from a different source from those discussed above, and is contained in Return [1847] No. 433. The relevant passage is as follows : " You will perceive, however, by the report of the brokers, that, judging from the samples, this variety must have greatly deteriorated, the value put on it being only $2\frac{3}{4}d$ to $3d$ per lb., ' when got up in the usual way and free from leaf '. The specimen is stated to be ' poor, and to have no appearance of good cotton ' ".

Mr. Finnie's report on five of Capt. Abbott's samples is particularly valuable in estimating the value of Malwa cotton relative to that of Nimar and Berar. The sample of *Nurma*, with which he expressed disappointment, he stated " is not better than some of the common cotton received from Malwa ". Of the common cotton of Nimar and Berar, on the other hand, he said that it fulfilled his expectations of *Nurma* cotton, and was " not inferior to good ' Uplands ' or New Orleans cotton ". From this it is clear that he considered Malwa cotton was usually definitely inferior to that of Nimar. This is confirmed by the fact that out of fifty-nine samples from Malwa, only one was valued by him as equal to " pretty good New Orleans ". That exceptionally good cotton was produced in fair quantity in places in Malwa is shown by the fact [reported by Royle{Return (1847) No. 22}], that Sironj cotton was exported to Dacca for the manufacture of fine muslins and sold there for about the same price as the local product.

Concerning *M'hahlie* cotton, opinions differed greatly. The most reasonable conclusion is that the two samples valued in Liverpool bore little relation to the three collected by Capt. Abbott. From Mr. Finnie's method of judging the Malwa cottons it is fair to conclude that he distinguished carefully between the intrinsic value of a staple and the actual condition in which it was presented for sale, while the Chamber of Commerce in Bombay naturally marked down heavily a sample which had suffered from inferior preparation. As Capt. Abbott stated that M'hahlie was normally never cleaned in Nimar, but sent to Chanderi in the pod, his samples are not likely to have been very well put up. Surgeon Irvine's opinion remains to be considered, but he was evidently an enthusiast for American cotton, with only an amateur's knowledge of quality, while Mr. Finnie was an experienced cotton planter, and presumably much better qualified to value it. It may then be concluded that a few villages in Nimar produced at that time a cotton of quite exceptional quality, while the bulk of the Nimar crop was of similar quality to the *Berari* cotton of Amraoti, and distinctly superior to the average of Malwa cotton.

Two further points of interest may be mentioned with regard to the cottons of Central India at that time. Bell [1843] reported on " Further particulars regarding Berareea cotton ". He sent an agent to Chanderi to buy some *Berareea kapas* in order to be sure of obtaining unmixed seed. The seed was cleaned and sown in his own garden under his personal supervision. He recorded : " It may be worth noticing that there are certainly three, and I suspect five descriptions of cotton in the field, all sprung from seed extracted from the same parcel of *kuppas*. The shape of the leaf, of the plants, and the flower all differ, and they certainly cannot be identical ". Hamilton [1849] gave ginning outturns of *kapas* from Malwa (Mehidpur and Ratlam) and Nimar. Calculated as percentages, the returns at the three centres were 26, 28, and 25 respectively. Abbott [*loc. cit.*] for Nimar gives outturns which appear to represent ginning percentages of 31 for M'hahlie and 25 for the common (*Guandailah*, or *Berari*) cotton.

Regarding the relative extent of cotton cultivation in Malwa and Nimar, Sir C. M. Wade [Return (1847) No. 144] referring to an enquiry concerning *Nurma* cotton stated, " the question was more interesting to that part of the country (Nimar), as it affected a staple article of its commerce, than to Malwa generally, in which cotton does not appear to succeed, and is but partially grown ". To this quotation may be added acreage figures (to the nearest 1,000 acres) for cotton cultivation in the Indore State in 1886, 1909, and 1930.

Year	Acreage	Source
1886-7	51,000	Watt [1890]
1909-10	290,000	Agric. Statistics of India [1915]
1930-1	625,000	Agric. Statistics of India [1934]

There has therefore been a twelve-fold increase in the area of cotton in the State in less than fifty years, which must have involved an enormous change in agricultural practice.

To the cottons of Rajputana, two references are made in the Return [1847]. In a Memorandum by R. C. N. Hamilton, Esq. [Return (1847) No. 73], it is recorded that " The cotton (grown from Mexican seed) was pronounced very superior to all in the native market at Agra.........., except Tonk, ... ". " The Tonk cotton though longer in the staple, perhaps, was said to have suffered from want of rain ". In extracts from Mr. Finnie's Journal [Return (1847) No. 148] it is stated that the largest supplies of cotton in the Agra market came from Jaipur and the neighbouring areas, but it was " of miserable quality ". It appears, therefore, that though Tonk produced good cotton at that time, neighbouring areas produced then, as now, only an inferior article.

III. Census

In order to collect adequate material for a statistical examination of the present composition of the cotton crops of Central India, it was decided to take a series of samples from cultivators' fields at about five-mile intervals on all roads leading out of Indore. The census was extended as far as the Nerbudda river on the south, the Kukshi Mahal of Dhar State on the west, the Nemawar district of Indore State on the east, and the Neemuch-Jhalrapatan road on the north. Advantage was also taken of an examination of the cotton crops of Alwar, Jaipur, Tonk and Bundi States to collect similar data in that part of Rajputana. Fig. 1 is a map of the Central India area studied, on which the distribution of the samples has been marked.

In sampling, the cotton field nearest to the mile stone was entered, and from 100 to 200 plants in a row chosen at random were classified into Upland, and narrow- and broad-leaved *desi*. Not all plants flower on any one day, and it was therefore necessary to take an independent sample of 100-200 *desi* plants on which there were flowers and classify them for leaf-shape and flower colour. It was feared that some bias might be introduced into the flower counts by the fact that white petals are shorter and more nearly covered by the bracteoles than yellow petals, and are therefore less easily seen. It was found, however, that there was an association between flower colour and leaf-shape, and a comparison of the leaf-shape proportions in the Upland *vs. desi* counts and in the flower counts was therefore used as a check against bias in the latter. It was found that the percentage of broads in row counts was lower by 2·01 than in flower counts, with a standard error of the difference of 1·00. The difference must therefore be regarded as significant, and indicates a slight, but definite, bias in the flower counts, resulting in an overestimate of the proportion of yellows by one to three per cent.

The area covered can be classified on geographical and ecological considerations into six tracts :—

(1) Rajputana, including data from Alwar, Jaipur, Tonk and Bundi.

(2) North Malwa, including data from Partabgarh, Sitamau, Rampura-Bhanpura (Indore), Jhalawar and Shajapur (Gwalior) (23°45′ to 24°40′ N.)

(3) Malwa, including data from Indore, Dhar, Ratlam, and Ujjain (Gwalior) (south of 23°45′ N. to the Vindhyas).

(4) Ghats, including data from cultivation in the Vindhyan valleys and slopes, in Indore and Dhar States, and Bagli Estate.

(5) Nimar, including data from the Nerbudda plain in Indore, Dhar and Barwani States.

(6) Nemawar, including data from the Nemawar district (Indore).

The geography of all tracts except Rajputana will be clear from the map.

In Table I are given the percentages of Upland cotton recorded in samples from the different tracts, and in Table II the four types of *desi* as percentages of the total *desi* component (*i.e.*, exclusive of Uplands). Analysis in this manner was necessary since flower counts had to be made separately from the count of Upland *vs. desi*. Where a direct comparison is desirable between Upland and one of the *desi* components separate summaries are given in the text of the proportions of Uplands and narrow- and broad-leaved *desi* in row counts, and conclusions are drawn from them.

TABLE I

Frequency arrays of percentage of G. hirsutum

Tract	Mean percentages																				Total	Mean	σ
	2·5	7·5	12·5	17·5	22·5	27·5	32·5	37·5	42·5	47·5	52·5	57·5	62·5	67·5	72·5	77·5	82·5	87·5	92·5	97·5			
Rajputana	33	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	34	0·3	1·78
North Malwa	1	6	3	2	1	1	1	1	1	...	...	1	...	...	...	...	...	...	...	...	19	18·5	15·27
Malwa	...	...	...	...	...	2	1	5	1	4	7	12	9	5	5	2	2	1	1	2	59	59·7	15·83
Ghats	1	3	6	1	5	1	2	2	2	...	...	...	1*	...	...	2*	...	...	...	...	26	27·0	20·06
Nimar	3	3	1	1	3	2	2	2	1	...	...	...	...	...	...	...	...	...	...	...	18	20·0	13·18
Nemawar . .	4	2	2	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	9	7·4	5·37

* Samples from the borders of the Nisarpur *Malan* tract

TABLE II

Frequency arrays of desi *types as percentages of all* G. arboreum *var.* neglectum

Area	*Desi* types	Mean percentages																				Total	Mean	σ
		2·5	7·5	12·5	17·5	22·5	27·5	32·5	37·5	42·5	47·5	52·5	57·5	62·5	67·5	72·5	77·5	82·5	87·5	92·5	97·5			
Rajputana	*Verum*	3	9	5	4	1	3	...	...	1	...	...	...	...	...	...	...	...	...	...	...	26	13·9	9·21
	Malvense	...	1	...	...	...	2	4	5	3	3	2	1	1	1	2	...	...	...	1	...	26	45·7	17·68
	Roseum	3	3	2	...	3	...	4	5	3	1	...	1	1	...	...	...	...	...	...	...	26	28·3	17·53
	Cutchicum	6	8	6	1	2	1	...	1	1	...	...	...	...	...	...	...	...	...	...	...	26	12·0	11·11
Malwa	*Verum*	1	...	5	7	14	11	4	1	2	...	...	...	...	...	...	...	...	...	...	...	45	23·3	7·79
	Malvense	...	...	...	...	...	...	1	...	1	...	9	3	5	12	9	4	1	...	...	...	45	64·5	11·79
	Roseum	13	15	12	2	1	...	1	...	...	1	...	...	...	...	...	...	...	...	...	...	45	10·0	8·60
	Cutchicum	40	3	2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	45	2·2	2·98
Ghats	*Verum*	...	1	5	6	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	13	15·1	4·70
	Malvense	...	...	...	...	...	4	2	2	2	...	...	...	1	1	1	...	...	...	...	...	13	41·5	15·70
	Roseum	2	...	1	...	1	...	...	...	3	4	...	2	...	...	...	...	...	...	...	...	13	36·4	19·60
	Cutchicum	5	5	2	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	13	7·0	4·71
Nimar	*Verum*	2	5	2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	9	7·9	3·19
	Malvense	...	1	3	2	1	1	...	...	1	...	...	...	...	...	...	...	...	...	...	...	9	19·2	9·92
	Roseum	...	...	...	...	...	...	...	1	...	...	...	1	1	2	...	3	1	...	...	...	9	67·9	13·92
	Cutchicum	5	3	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	9	4·9	2·96
Nemawar	*Verum*	...	...	...	5	...	1	2	...	...	...	...	...	...	...	...	...	...	...	...	...	8	22·7	6·40
	Malvense	...	...	...	...	1	...	...	...	...	1	...	...	2	1	1	1	1	...	...	...	8	61·6	19·14
	Roseum	5	1	...	...	...	...	...	1	...	...	...	1	...	...	...	...	...	...	...	...	8	14·2	20·71
	Cutchicum	8	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	8	1·5	0·89

Upland plants were only found in a single sample in Rajputana. This sample was taken in Dausa tahsil of Jaipur State and contained 10·4 per cent of Upland. It was probably the result of distribution on a small scale of a Malwa Upland strain by the Jaipur Department of Agriculture.

With the exception of the above and of a small area in Khetri Thikana of Jaipur, the source of seed in all areas included in the Rajputana survey was the cultivators' own crops, hand ginned in their homes, and no introduction had taken place in living memory. In Khetri Thikana some so-called *Mollisoni* seed obtained from the Punjab has been distributed during the last few years. One crop from Punjab seed was sampled, and is included in Table II. Its composition was as follows :—

Verum	*Malvense*	*Roseum*	*Cutchicum*
5·3	9·1	41·7	43·9

As only the component recorded as *Cutchicum* agrees with the generally accepted characteristics of *Mollisoni*, the seed imported must have been extremely mixed. Omitting this sample and allowing for the influence of Punjab seed in raising the proportion of white-flowered types in some other samples from Khetri, the Rajputana samples still indicate considerable variability in crop composition. In particular the proportion of *Roseum* fluctuated widely. When the samples were taken it was noted that different cultivators, often in the same village, had different preferences, and exercised some selection among the crop components in what was reserved for seed. The main outline of the situation is adequately represented by the mean values, however, and it is clear that in general the *Malvense* type predominated, contributing a little less than half the crop. *Roseum* contributed in one group of samples from five to ten per cent of the crop, and in the rest about twenty to forty-five per cent. *Verum* and *Cutchicum* averaged about twelve per cent each.

In north Malwa, Upland was present to the extent of eighteen per cent of the crop on the average. Only one flower count is available as it was late in the season when the tract was visited. From the proportion of broad to narrow-leaved types in the row samples, it appears that the composition of the *desi* component was more similar to that of Rajputana than to that of Malwa.

Percentage of broad-leaved plants in the *desi* component—

Rajputana	57·1
North Malwa	55·3
Malwa	65·1

The encouragement given to the growing of Upland, and the distribution of Cambodia seed, by Coventry and others—notably the Dhar State Department of Agriculture—have given rise in Malwa to a distinct Malwa Upland crop, grown chiefly on *adhan* lands (village lands, heavily manured) where well or tank irrigation is available to supplement rainfall if necessary. Census samples in Malwa were not usually taken in such fields, but occasionally one happened to be the nearest to the mile stone and was accordingly examined. They appear in Table I as fields with ninety per cent or more of Upland. In some localities, as for example Badnawar where considerable areas of land are commanded by tanks, the Malwa Upland crop is of some importance. Even in fields of supposedly *desi* cotton, however, on an average Upland forms nearly sixty per cent of the crop.

So far as the composition of the *desi* component is concerned, Nemawar is closely similar to Malwa, and its cotton ranks as Malvi in the Indore market. The two may conveniently be considered together. Nemawar is a somewhat isolated district of the Indore State lying between the Vindhyas and the Nerbudda. It is cut off from other parts of the Nimar valley by natural barriers of hills and rivers and by State and British India boundaries. These together have resulted in its only important outlet being by road to Malwa. In contrast to Malwa, Upland has not invaded it to any great extent, and is only present as about seven per cent of the crop.

It was through the seed-distributing activities of the gin owners of Malwa and Nemawar that it was believed that the influx of inferior *Roseum* seed took place. A prominent cotton buyer of one of the Indore mills expressed the opinion (in conversation) that ginneries have from time to time introduced *Roseum* seed in considerable bulk into various parts of the Malwa and Nemawar tracts in the hope of improving ginning percentage, and that the result has been a serious deterioration in quality in the area affected, which lasted a few years and was followed by a gradual recovery. In some of the few cases where high proportions of *Roseum* and low proportions of *Malvense* were observed, there were other indications that the crop was from introduced seed, and that Nimar seed is sometimes introduced is unquestionable. The samples show, however, that on the whole, *Roseum* is comparatively rare, and that crops containing considerable proportions of it are only occasionally found. In both Malwa and Nemawar *Malvense* contributes over sixty per cent of the *desi* component, and *Verum* over twenty per cent, leaving little more than ten per cent for *Roseum*, and only two per cent for *Cutchicum*. The Indore mills grade the *kapas* which they purchase before ginning, and only return to the villages seed from the better grades. There is no doubt that one effect of this policy is to augment the Upland component in the local crops, since, other things being equal, lots containing a high proportion of Upland will be valued more highly in the mill than those with less. Apart from this, certain villages have a reputation for good cotton, and it is a regular mill practice to see that their own seed is returned to them in order to perpetuate what is supposed to be a superior strain. Eight of the Malwa samples were taken from crops in villages with a good reputation for their cotton, and below is given an analysis of the crop components in the good villages and in the rest of Malwa (Table III).

TABLE III

Comparison of crop composition in good villages and the rest of Malwa

Component	Good villages	Rest of Malwa	Difference	S. E. of difference
Upland as percentage of the whole crop				
	56·0	60·6	—4·6	15·22
Neglectum types as percentages of the *desi* component				
Verum	27·3	22·5	+4·8	2·48
Malvense	62·8	64·9	—2·1	3·87
Roseum	8·4	10·3	—1·9	1·84
Cutchicum	1·5	2·3	—0·8	0·74

The only difference of any importance between good villages and the rest is in the proportion of *Verum*, which was higher in good villages by an amount almost twice as great as its standard error. On the whole crop (including Uplands) this represents a difference of not more than two per cent, and certainly cannot be regarded as being responsible for the reputation of the good villages. Inspection of the condition of the crops at the time of sampling led to the conclusion that the good villages owe their superiority to the nature of their land and not to the strain of cotton they grow. Throughout Malwa the Upland component suffers much more severely than the *desi* from adverse weather and soil conditions, and therefore serves as a good index of the condition of the crop. In the villages with a good reputation the Upland component was better grown and suffered less from red-leaf and leaf-roll than in other villages.

In Nimar there has been some seed introduction from outside. The most important has been the introduction of *Roseum* from Khandesh by the Dhar State Agricultural Department. There have also been occasional introductions in recent years of Banilla from the Khandesh and Verum 262 from the Central Provinces, but these have not been on sufficient scale to affect the crop composition. The cultivators in the areas of Nimar included in the present study (Indore, Dhar, and Barwani States) may be divided into two groups according to their methods of acquiring seed for sowing ; (*a*) cultivators who either get their *kapas* ginned, and keep their own seed, or who buy seed from those who do, and (*b*) cultivators who buy their seed from a ginnery or from a *bania*. The former group includes the more progressive men and those who are better off. The latter is made up of those who are too indifferent, or too heavily indebted, to select their seed. In sampling the Nimar fields it was usually possible to say after a rough inspection to which class the owner belonged. Those in the first group usually had crops containing ten per cent or less of *Malan* (Upland) and seventy per cent or more of narrow-leaved *desi*. In a number of cases they traced their stock direct to *Roseum* seed imported three or four years previously. Those in the second group usually had crops with twenty to forty per cent of *Malan*, and less than fifty per cent of narrow-leaved *desi*.

In Table IV are given data from row counts in the Nimar samples, showing percentages of *Malan*, and narrow- and broad-leaved *desi*. The broad-leaved *desi* may be taken as almost all *Malvense*, but of the narrow-leaved, about nine-tenths were probably *Roseum*, and the rest *Verum*.

TABLE IV

Percentages of Upland and narrow- and broad-leaved desi *in Nimar row samples*

Sample number	Upland	*Desi*		Remarks
		Broad	Narrow	
B. A. 55 . .	0·0	13·4	88·6	3 years from *Roseum* stock.
I. B. M. 66 .	1·3	23·2	75·5	
B. A. 49 . .	3·3	21·7	75·0	*Roseum* kept "pure".
I. B. M. 65 .	6·9	7·8	85·3	
I. B. M. 64 .	6·9	15·6	77·5	
B. A. 52 . .	7·1	17·7	75·2	Thikri. Centre of *Roseum* distribution.
B. A. 54 . .	11·2	22·8	67·0	
I. B. M. 59 .	15·9	14·1	70·0	
I. B. M. 60 .	24·4	28·9	46·7	
I. B. M. 61 .	24·9	19·0	56·1	
I. B. M. 63 .	26·0	30·7	43·3	
I. B. M. 62 .	29·2	21·7	49·1	
B. A. 48 . .	31·4	17·9	50·7	
B. A. 53 . .	32·3	30·1	37·6	
B. A. 50 . .	33·8	18·4	47·8	
B. A. 56 . .	37·2	36·4	26·4	
B. A. 51 . .	41·0	8·5	50·5	

The samples fall fairly distinctly into two classes, one with high *Malan* and relatively low *Roseum*, and the other with low *Malan* and high *Roseum*. The percentage of *Malvense* bears little relation to the main grouping.

When questioned, better class cultivators usually stated that they did not like much *Malan* in their fields, and they liked a high proportion of the *Roseum* type, as it gave a good yield and a good ginning percentage. Ginners, on the other hand, expressed a very definite preference for a mixture containing a high proportion of *Malan*, as the ginned cotton was then of better quality, and more easily sold.

The more independent cultivators have been able to some extent to maintain a distinct type of cotton in spite of ginners' preferences. Below is given the composition of samples from three crops grown from *Roseum* seed three generations removed from the original introduction :—

Upland as percentage of total	Percentages of total *desi* component			
	Verum	*Malvense*	*Roseum*	*Cutchicum*
2·5	8·7	14·7	75·7	0·9
7·1	6·9	9·9	79·5	3·9
0·0	3·4	10·3	84·5	1·7

In two cases about twenty-five per cent of the crop and in the third about fifteen per cent were impurities.

It remains to consider the relative proportions of *Malvense* and *Verum.* Neither of these types has been the object of conscious human selection, and their proportions may be taken to indicate their relative natural selection values. *Verum* contributed about eight, and *Malvense* nineteen per cent of the *desi* crop, indicating a very marked advantage to *Malvense. Cutchicum* contributed about five per cent, and as in other tracts is of negligible importance.

The samples included in the Ghats tract do not form a natural homogeneous group. One sample was taken on the road between Malwa and Nemawar and, as might have been expected, gave a composition closely similar to that of Nemawar, with 9·5 per cent of Upland, and in the *desi* component, 25·0 per cent *Verum*, 70·8 per cent *Malvense*, 1·7 per cent *Roseum*, and 2·5 per cent *Cutchicum.*

Included in the Ghat samples are also samples from the Kukshi Mahal of Dhar State and the Nisarpur pergana of Indore State, which lie in isolated broken country between the Vindhyas and the Nerbudda, with the river forming the boundary between them and Barwani State. Four samples in which flower counts were made and seven other row samples were taken in this district. Below are given percentages of Upland in the eleven samples—

3·0
9·9
12·8
20·8
21·1
33·9
36·3
40·9
64·6
76·8
77·0

The last three, with very high percentages of Upland, were taken on the edge of the Nisarpur district, where *Malan* is grown almost pure. This small island of pure Upland cultivation is remarkable, in that in the rest of Nimar pure crops of *Malan* fail miserably, even though the type thrives when grown in mixtures up to as much as forty per cent of Upland.

The proportions of the *desi* component in the four Kukshi samples from which flower counts are available were as under :—

Verum	*Malvense*	*Roseum*	*Cutchicum*
16·7	44·4	25·0	13·9
15·8	62·0	11·1	11·1
13·5	41·9	41·9	2·7
11·4	69·6	2·5	16·5

These four samples, and the Malwa-Nemawar road sample account for all the low *Roseum* and high *Malvense* samples in the Ghats summary in Table II, and for the corresponding high Upland samples in Table I. The remaining samples form a compact group intermediate in composition between the crops of Malwa and Nimar in regard to the *desi* component, and similar to Nimar in regard to the Upland component (about twenty per cent).

One striking feature of the samples from all tracts remains for comment. It is the strong association which exists between leaf-shape and corolla-colour. χ^2 for independence was as follows in the five tracts : Rajputana 21·7, Malwa 15·3, Nemawar 22·5, Ghats 32·2, Nimar 44·5. It is known [Hutchinson, 1934] that there is no linkage between the two factor pairs, so that it is not possible to suppose that linkage is responsible. The existence of the association in Rajputana, where no seed introduction has taken place, disposes of the possibility that it might be due to the lag between invasion of a particular type and the attainment of equilibrium by crossing and segregation between invader and native. There remains as the only possible explanation the conclusion that broad-leaved, yellow-flowered and narrow-leaved, white-flowered types are at a selective advantage under all conditions studied over narrow-leaved, yellow-flowered and broad-leaved, white-flowered types.

IV. Crop analysis

The assumption that high ginning percentage and coarseness are associated with white flower, and in particular with the white-flowered narrow-leaved type known as *Roseum*, appears to be universal among cotton botanists in India, but we are not aware of any published data in support of it. The theory of the adulteration of *Malvi* cotton with *Roseum*, to test which this investigation was undertaken, would be disproved equally well by a demonstration that *Roseum* is not inferior, as by proof that it has not, in fact, penetrated into Malwa. It was therefore considered desirable to study the lint and yield characters of the four *desi* components of the crop.

The characters studied were—

(1) Mean yield (given in grms. per plant in Tables V-VIII and Table IX for 1933-34, and in grms. per plot in Table IX for 1934-35).

(2) Lint-index and seed-weight (in grms. per 100 seeds).

(3) Halo-length (mean maximum length on ten seeds in mm.).

(4) Fineness (mean hair diameter on 50 hairs per plant after swelling in eighteen per cent caustic soda. See Clavert and Harland [1924]. Data given in units of 0·00357 mm.).

In order to distinguish between any fortuitous association of lint characters with morphological characters, and genetic linkage, two types of material were studied. First, an analysis was made of lint characters in the four phenotypic classes in F_2 families segregating for leaf-shape and corolla-colour. Any differences occurring in such material between classes must be due to genes in the leaf-shape or corolla-colour chromosomes. Secondly, plants of the four phenotypic classes were chosen in 1933-34 from a field of mass-selected Malvi. The strain had been grown for about seven years on the Institute farm, and up to and including the 1931-32 season, it was carefully rogued of all narrow-leaved and white-flowered plants. Accidental admixture was avoided as carefully as possible, but either a slight amount of contamination from unselected Malvi must have occurred, or the roguing was not completely efficient. Any genetic differences between the four classes will be present in such material, but differences due to genes in other chromosomes will not necessarily be distributed at random and may result in differences between classes not exhibited by the data from the F_2 families.

In choosing the form of the analyses, it was thought that the differences between factor-pairs were likely to be of primary importance, and the differences between the four classes were partitioned in three ways: into (1) differences between flower-colour classes, (2) differences between leaf-shape classes, and (3) interaction, which represents the extent to which the effect of one factor is affected by the presence of a member of the other factor pair.

In Tables V to VIII are given data for lint characters in four F_2 families from sister F_1 plants of Bani × C. 520. Leaf-shape, corolla-colour and interaction classes are given separately.

TABLE V

Lint characters of phenotypic classes in Bani × C. 520 F_2 family 871

Yellow flower *vs.* white flower

Character	Y	y	Difference	*P*
Yield	19·86	13·13	+6·73	<0·01
Halo-length	25·77	25·78	—0·01	>0·9
Ginning percentage	36·07	36·51	—0·44	0·6
Lint index	2·81	2·77	+0·04	0·8
S. H. diameter	13·42	12·89	+0·53	0·2

Narrow leaf *vs*. broad leaf

Character	L	l	Difference	*P*
Yield	18·33	19·70	—1·37	0·7
Halo-length	25·75	25·81	—0·06	>0·9
Ginning percentage	36·31	35·64	+0·67	0·4
Lint index	2·80	2·68	+0·12	0·5
S. H. diameter	13·31	13·38	—0·07	0·9

Interaction

Character	YL+yl	Yl+yL	Difference	*P*
Yield	19·28	17·46	+1·82	0·5
Halo-length	25·77	25·76	+0·01	>0·9
Ginning percentage	36·43	35·62	−0·81	0·2
Lint index	2·81	2·79	+0·02	0·9
S. H. diameter	13·53	12·95	+0·58	0·2

TABLE VI

Lint characters of phenotypic classes in Bani × C. 520 F_2 family 872

Yellow flower *vs*. white flower

Character	Y	y	Difference	*P*
Yield	18·37	14·72	+3·65	0·05
Halo-length	25·41	25·55	+0·14	0·8
Ginning percentage	36·60	37·47	—0·87	0·5
Lint index	3·02	2·81	+0·21	0·3
S. H. diameter	13·27	13·23	+0·04	>0·9

Narrow leaf *vs*. broad leaf

Character	L	l	Difference	*P*
Yield	18·47	16·54	+1·93	0·3
Halo-length	25·22	25·99	—0·77	0·05
Ginning percentage	36·65	36·85	—0·20	0·8
Lint index	2·91	3·21	—0·30	0·02
S. H. diameter	13·31	13·13	+0·18	0·6

Interaction

Character	**YL+yl**	**Yl+yL**	Difference	*P*
Yield	18·47	15·94	+2·88	0·1
Halo-length	25·10	26·05	−0·95	<0·01
Ginning percentage	35·48	36·98	−1·50	0·1
Lint index	2·97	3·05	−0·08	0·6
S. H. diameter	12·87	13·28	−0·41	0·4

TABLE VII

Lint characters of phenotypic classes in Bani × C. 520 F_2 family 873

Yellow flower *vs.* white flower

Character	**Y**	**y**	Difference	*P*
Yield	18·20	18·45	−0·25	0·9
Halo-length	24·98	24·90	+0·08	0·9
Ginning percentage	35·94	36·55	−0·61	0·3
Lint index	2·86	2·79	+0·10	0·4
S. H. diameter	13·62	13·66	−0·04	>0·9

Narrow leaf *vs.* broad leaf

Character	**L**	**l**	Difference	*P*
Yield	18·98	16·77	+2·21	0·4
Halo-length	24·72	25·30	−0·58	0·3
Ginning percentage	36·61	34·85	+1·76	0·1
Lint index	2·81	2·93	−0·12	0·3
S. H. diameter	13·49	13·88	−0·39	0·3

Interaction

Character	**YL+yl**	**Yl+yL**	Difference	*P*
Yield	18·81	17·43	+1·38	0·4
Halo-length	24·76	25·14	−0·38	0·4
Ginning percentage	36·57	35·26	+1·31	0·1
Lint index	2·81	2·92	−0·11	0·3
S. H. diameter	13·44	13·88	−0·44	0·2

TABLE VIII

Lint characters of phenotypic classes in Bani × C. 520 F_2 family 874

Yellow flower *vs.* white flower

Character	**Y**	**y**	Difference	*P*
Yield	18·69	17·46	+1·23	0·01
Halo-length	25·15	25·43	−0·28	0·5
Ginning percentage . .	37·00	37·38	−0·38	0·6
Lint index	2·88	3·06	−0·18	0·3
S. H. diameter . . .	13·45	14·19	−0·76	0·2

Narrow leaf *vs.* broad leaf

Character	**L**	**l**	Difference	*P*
Yield	19·09	16·56	+2·53	0·2
Halo-length	25·10	25·52	−0·42	0·3
Ginning percentage . .	37·11	37·03	+0·08	>0·9
Lint index	2·96	2·82	+0·14	0·4
S. H. diameter . . .	13·74	13·78	−0·04	0·3

Interaction

Character	**YL+yl**	**Yl+yL**	Difference	*P*
Yield	19·62	16·62	+3·0	0·05
Halo-length	25·05	25·45	−0·40	0·3
Ginning percentage . .	36·91	37·34	−0·43	0·7
Lint index	2·86	3·01	−0·15	0·3
S. H. diameter . . .	13·68	13·86	−0·14	0·7

In family 873 there were no significant differences between classes in any of the characters studied. In families 871, 872 and 874, yellow-flowered classes yielded significantly more than white flowered. From the interaction data, it appears that in family 874 the difference was due to the **YL** class only, whereas in 871 and 872, both **Y** classes contributed.

In family 872 narrow-leaved plants had a significantly lower mean halo-length and mean lint index than broad-leaved plants. The **Yl+yL** classes together had a significantly higher halo-length than the **YL+yl** classes showing that the greater halo-length of broad-leaved plants was due to the broad-leaved, yellow-flowered class only.

It is clear that genes for high yield occur in the corolla-colour chromosome and for long lint and high lint index in the leaf-shape chromosome but they are only linked, and not completely coupled with corolla-colour and leaf-shape respectively, as the Bani parent, from which they must have been derived, was heterozygous for them. Any differences, therefore, which may be found between classes in the material obtained from mass-selected Malvi must be regarded as most probably due to fortuitous association of genes which natural crossing has not been sufficient to equalise. Linked genes occur, but there is no evidence of complete coupling, or of any direct effect of the leaf-shape and corolla-colour genes on lint characters.

An attempt was made to select from mass Malvi twenty-five plants of each phenotype, but the *Roseum* and *Cutchicum* types were so rare that only twenty-three *Roseum* and nineteen *Cutchicum* plants were obtained. Owing to accidents after labelling, one *Roseum* and four *Cutchicum* plants were lost. The *kapas* of each plant was collected and examined. In 1934-35 progenies were grown from all plants. No heterozygotes were to be expected in the doubly recessive *Cutchicum*. *Malvense* comprised such an overwhelming proportion of the crop that out-pollination of any type would almost certainly involve *Malvense* pollen. Consequently very few heterozygotes would be expected in *Malvense*, and practically none in *Roseum*, as *Roseum* × *Malvense* would give *Verum*. *Verum*, therefore, is the only class in which any considerable proportion of heterozygotes is to be expected, and even among *Verums*, corolla-colour heterozygotes should occur in only very small numbers because of the very low proportion of white-flowered plants in the population. Of the twenty-five *Verum* plants eight were heterozygous for leaf-shape. There were no flower-colour heterozygotes in any class and no other leaf-shape heterozygotes.

In the 1934-35 season a test was laid out at Indore consisting of nineteen randomised blocks, each containing four plots, one each of *Verum, Malvense, Roseum,* and *Cutchicum*. Each plot consisted of a single row, 12 ft. long. Rows were one foot apart. Each row was sown with thirty seeds from a single plant of the 1933-34 samples. Nineteen plants of each type, therefore, were included in the experiment and each one occurred once only. By thus randomising different progenies in different blocks, progeny differences were confounded with block differences, and differences between different progenies of the same type contributed, together with environmental differences, to the estimate of error which was used in testing the significance of differences between types. This method was better suited to the object in view than comparing the behaviour of a single pure strain of each type. The latter method would have given a lower error, but the essential point of the comparison was to test whether differences between types were greater than those occurring within types, and it was therefore necessary to include in the estimate of error a contribution from differences between progenies of the same type. At flowering time it was discovered that of the nineteen types sown as *Cutchicum* three were *Malvense* and one was *Roseum*. Enquiry led to the conclusion that some of the labels which had been attached to the sample plants in 1933-34, and had blown down, had been attached to other plants by casual labourers. The four *Cutchicum* plots thus affected were discarded. The data were reduced by the analysis of variance and for the discarded plots, values were calculated by the "missing plot technique" [Yates, 1933].

Mean values, differences, and significances of differences between flower-colour, leaf-shape, and interaction classes are given in Table IX for the 1933-34 plants and their 1934-35 offspring.

TABLE IX

Comparison of lint characters of 1933-34 samples and 1934-35 progenies

Character	1933-34 plants				1934-35 progenies			
Yellow flower *vs.* white flower								
	Y	y	Difference	*P*	Y	y	Difference	*P*
Yield	26·9	14·2	+12·7	<0·01	92·0	44·3	+47·7	0·05
Halo-length	22·0	21·4	+0·6	0·03	23·0	21·7	+1·3	0·05
Ginning per cent	27·5	31·2	—3·7	<0·01	26·5	33·0	—6·5	0·05
Lint index	2·06	2·75	—0·69	<0·01	1·99	2·60	—0·61	0·05
Seed weight	5·42	5·86	—0·44	0·01	5·45	5·33	+0·12	large
Swollen hair diameter	6·67	8·06	—1·39	<0·01	6·77	8·14	—1·37	0·05
Narrow leaf *vs.* broad leaf								
	L	l	Difference	*P*	L	l	Difference	*P*
Yield	18·2	25·4	—7·2	<0·01	67·3	69·0	—1·7	large
Halo-length	21·8	21·7	+0·1	large	22·2	22·5	—0·3	large
Ginning per cent	29·7	28·3	+1·4	0·1	30·0	29·4	+0·6	large
Lint index	2·48	2·20	+0·28	0·01	2·34	2·25	+0·09	large
Seed weight	5·68	5·52	+0·16	0·3	5·37	5·34	+0·03	large
Swollen hair diameter	7·31	7·17	+0·14	0·18	7·48	7·46	+0·02	large
Interaction								
	YL+ yl	Yl+ yL	Difference	*P*	YL+ yl	Yl+ yL	Difference	*P*
Yield	17·9	24·8	—6·9	<0·01	72·4	63·8	+8·6	large
Halo-length	21·8	21·7	+0·1	large	22·2	22·5	—0·3	large
Ginning per cent	28·3	29·7	—1·4	0·1	29·4	30·1	—0·7	large
Lint index	2·12	2·55	—0·43	<0·01	2·20	2·39	—0·19	large
Seed weight	5·28	5·86	—0·58	<0·01	5·26	5·45	—0·19	large
Swollen hair diameter	6·28	7·25	—0·97	<0·01	7·32	7·61	—0·29	>0·05

In both years yellow-flowered types gave double the yield of white-flowered types. It is obvious that with such a yield difference, when *Roseum* is introduced into Malwa, it must be very rapidly eliminated by natural selection. Yellow flowered types were superior in lint-length by 0·6 mm. among parents and by 1·3 mm. among progenies. They had very much finer lint also, having swollen hair diameters almost 1·4 units, or about seventeen per cent, less than white-flowered types. The differences in these two characters easily account for the superiority of these types of *Verum* and *Malvense* in spinning value. Concurrently with the higher swollen hair diameter, the ginning percentage and lint index of the white-flowered types exceeded those of the yellow-flowered by considerable amounts. The parallelism between lint index, ginning percentage, and swollen hair diameter explain the reason for the association between high ginning percentage and low spinning value so common in Indian cottons. In cases such as this, ginning percentage is high in those types which have heavy—and therefore coarse—hairs.

In leaf-shape, broad-leaved parents gave significantly higher yields and lower lint indices than narrow-leaved parents. These differences were not confirmed by the progenies. Homozygous and heterozygous *Verum* progenies were examined separately, as it was thought that the occurrence of *Malvense* plants in the heterozygous *Verum* plots might be responsible for the disappearance of the differences between narrow and broad classes. The means of homozygous and heterozygous families were practically identical, so that this possibility was ruled out. It appears that the effect of leaf-shape on the characteristics of the Malwa crop, if any, must be quite small.

The study of the effect of interaction between leaf-shape and flower-colour on yield and lint characters is of great interest on account of the fact that, in spite of the absence of linkage between the two factor pairs, the yellow-flowered broad-leaved, and white-flowered, narrow-leaved types were in great excess over their expected proportions in every tract studied. Among parents, the *Malvense* + *Roseum* pair gave significantly higher yields, lint indices, seed weights, and swollen hair diameters than the *Verum*+*Cutchicum* pair. Of these differences, only that in swollen hair diameter approached significance among progenies. The *Verum*, *Roseum*, and *Cutchicum* plants selected in 1933-34 were all isolated individuals growing in a large population of *Malvense*, and the competition effects are likely to have been different from those occurring in the 1934-35 experiment, where there was a row of each type in each block. It may be noted that the two types (*Malvense* and *Roseum*) which occur in the natural population in excess of the expected proportions were at an advantage in the single plant comparisons in just those factors likely to be most affected by competition, namely, yield, seed-weight, and lint index. While this is suggestive, it cannot be regarded as conclusive, and indicates the need for a definite study of competition effects.

Since the *Roseum* and *Cutchicum* plants selected from the mass-selected Malvi occurred widely scattered in a crop an overwhelming proportion of which was *Malvense*, the proportion of yellow-flowered plants among their offspring gave a very good estimate of the amount of natural crossing. Progenies were obtained from thirty-six plants of the two types, comprising 822 plants. In only three progenies were yellow-flowered plants recorded, eighteen such plants in all, or 2·2 per cent. In the three progenies there were eighty-three plants giving nearly twenty-two per cent natural crosses. Natural crossing is therefore of very little importance on the whole, but—as might be expected with insect-pollination—in the progenies of plants on which natural crossing has occurred, the proportion of natural hybrids is very much higher than in the population as a whole.

V. Discussion

The investigation here reported started from the current belief that serious deterioration had occurred in Malvi cotton as a result of the influx of inferior seed from Nimar and Mewar. This belief has been widely held, and was accepted by the Indian Cotton Committee [1919]. Systematic study has shown it to be without foundation, and in its place it has been possible to build up a well-documented account of the history and ecology of the cotton crop in Central India which will prove a valuable guide to those engaged upon its improvement.

The results obtained emphasise the great importance of scientific enquiry as a pre-requisite to the proper planning of a programme of agricultural improvement, and provide a striking demonstration of the unreliable and even misleading nature of popular opinion unsupported by statistical evidence.

The history of cotton in Central India has been confused by inadequate and incorrect quotation, and the original sources are not easily obtained *. A study of them, however, gives a clear picture of the former status of the crop and of recent changes in its distribution and composition. The botanical changes involved have been discussed elsewhere [Hutchinson, in press]. It was there shown that in response to the modern demands of the cotton trade, the old fine Oomras (*G. arboreum* var. *neglectum* forma *indica*) of the Central Provinces, Berar and Nimar were replaced by coarse, high-ginning types of *G. arboreum* var. *neglectum* forma *bengalensis*. This is the origin of the coarse Nimari cottons which were supposed to have been largely responsible for the deterioration of Malvi, and the demonstration of the fact that they are not indigenous in Nimar, and of the high value of the types they replaced, is sufficient to show that it is Nimar and not Malwa that has suffered most from deterioration, and is intrinsically the quality cotton tract. At the time the despatches included in the Return [1847] were written, Malwa was not considered to be a cotton-growing tract of any great importance.

---

* A copy of the Return on Cotton in India, published in 1847, and frequently referred to in the foregoing pages, is now in the possession of the Imperial Bureau of Plant Genetics, Cambridge, and is on permanent loan to the Library of the Institute of Plant Industry, Indore, Central India. It contains much valuable information on the early attempts to improve the cotton crop of India, and deserves to be more widely known.

The origin of the *neglectum* cottons of Malwa and Rajputana requires a little consideration. It was concluded [Hutchinson and Ghose, in press] that the northern limit of the distribution of forma *indica* was the Vindhyan range, and Malvi Oomras and Rajputana Bengals were therefore classed as forma *bengalensis*. Examination of fine selections made in crops of Rajputana Bengals have recently cast doubt on the validity of this assumption. Many of the selections have every appearance of being forma *indica* of the *Bani* type. This suggests that the coarse *bengalensis* of Rajputana also is an invader. Considerable further work is required on this problem, as it is a matter of considerable importance for the planning of cotton-breeding policy in northern India, whether or not the predominantly coarse *bengalensis* type is sufficiently variable to give rise to medium staple crops of the Malvi type.

The historical study of the cotton of Nimar, and the census of the existing crop in Rajputana shows that before the advent of modern ginneries, and in areas where they do not at present exist, individuals or groups of cultivators maintained strains which often differed markedly from those of their neighbours. In Nimar the fine *M'hahlie* was grown in two districts, specially for the fine spinners of Chanderi, and was successfully kept separate from the local *Berari*. In Rajputana, Tonk produced the best cotton in the Agra market, and neighbouring areas in Jaipur the worst, while Sironj supplied some of the raw material for the fine Dacca muslins. In the present crop the variation from field to field in crop composition is much greater in Rajputana than in the other areas studied, and this was found to be the result of the selection exercised by individual cultivators, all of whom maintain their own seed stocks. Ginnery mixing has resulted in a very uniform crop composition in Malwa, but in Nimar, intelligent cultivators still manage to exercise some control over the seed they sow. In all cases, however, the crop consists of a mixture of botanical types. Pure strains do not exist now in any part of the area studied and there is no evidence that they ever have done [Bell, 1843].

The failure of the inferior *bengalensis* types to invade Malwa is conclusively shown by a comparison of old and current opinions of the value of Malvi cotton, by the small proportions (about five per cent) in which white-flowered types occur in the crop and by the close similarity in crop composition between villages with a reputation for good quality cotton and the rest of Malwa. It may, of course, be argued that deterioration has occurred through natural crossing, and the demonstration that there is no necessary connection between lint characters and morphological characters shows that such deterioration is possible. The small percentage of immigrant types in the general population, the rapidity with which they appear to be eliminated by the selective effect of their yield disadvantage, and the very low rate of natural crossing, together minimise any infiltration of genes from outside.

Having shown that the current theories of the causes of deterioration of Malvi cotton are without foundation, it is of interest to enquire whether it is really a fact that deterioration has occurred. Apart from general statements unsupported by data of any kind, the only information we have been able to discover is that given by Messrs. Norris and Hartley for lea tests of Malvi and Nimari cottons spun to approximately 20's counts in the Indore Malwa Mills in 1914 and 1916-17 [Indian Cotton Committee. Evidence Vol. V, 1920]. By courtesy of a member of the staff of one of the Indore mills, it is possible to supplement this data with figures for the years 1921 and 1924-1934. All data were for lea tests on yarns spun to approximately 20's counts with approximately twenty turns per inch in 1914 and 1917 and 18·5 subsequently. Below are given mean lea strengths in lbs. for Malvi and Nimari separately for all years for which data are available.

Year	Nimari	Malvi
1914	60	68
1916-17	47	46
1921	36	55
1924	56	60
1925	55	60
1926	50	51
1927	47	52
1928	48	52
1929	49	56
1930	37	38
1931	33	36
1932	20	31
1933	20	35
1934	20	36

At first sight there appears to be very good evidence of serious deterioration in strength at 20's counts. Taking the data for the eleven years 1924-1934 for which annual means are available, it will be seen that they fall into three sharply demarcated groups : (1) 1924-1929 with Nimari varying from forty-nine to fifty-six and Malvi varying from fifty-one to sixty, the annual fluctuations being roughly parallel in the two types : (2) 1930 and 1931 with Nimari thirty-seven and thirty-three and Malvi thirty-eight and thirty-six, and (3) 1932-1934 with Nimari at twenty and Malvi from thirty-one to thirty-six. Malvi did not change between the second and third periods. The sharpness of the changes between groups strongly suggests a change in buying policy rather than a change in the crop, though a part of the recent change in the Nimar data may be due to the spread of the Khandesh *Roseum* introduced about 1929 by the Dhar State Department of Agriculture.

With this in mind the evidence of the Indore industry before the Indian Cotton Committee was further examined, and fresh enquiries were made from the mills in Indore and Ratlam concerning their opinions on recent changes in crop quality. A very large part of the deterioration complained of in 1917 was deterioration due to increasing contamination with foreign matter, either deliberate in ginneries, or due to carelessness in picking and storing. This is, of course, quite outside the scope of agricultural botany, and no analysis of changes in waste losses is included here.

The remaining portion of the deterioration complained of was said to have taken place over so short a period as to make it impossible to ascribe it to changes in crop composition, and it was probably due to seasonal fluctuations. The data on lea strengths support this interpretation. Allowing for the difference in twist, the whole of the drop in strength subsequent to 1914 had been made up by 1924.

This is in agreement with mill opinion in 1935, which was that the best lots of Malvi cotton are as good as they ever were, and in places (such as Ratlam and Badnawar) where Cambodia thrives, better cotton is obtainable now than in former years. Among ordinary and inferior samples great variation exists, and there have been cases of serious deterioration owing to the distribution of inferior seed. This can usually be traced to the activities of a particular ginnery, and in Malwa the effect tends to disappear, and the cotton regains its former quality in a few years. This testimony to the efficiency of natural selection in re-establishing the local type is striking. An instance of the same effect is to be found in the evidence of Mr. Davur before the Indian Cotton Committee [Evidence, Vol. V (Part II) 1920] on the survival of the *saloo* cotton of Aurangabad in the face of the introduction of *Roseum barat* from Khandesh.

The observation during the census work that cotton of superior quality is produced in villages with superior land has a considerable bearing on the problem of deterioration in Malwa. The twelve-fold increase in the area under cotton that has occurred in the past fifty years must have resulted in the spread of the crop from the lands for which it is well adapted to areas where it grows much less satisfactorily, and while the quality of the best lots should remain the same, the amount and variability of inferior cotton would be expected to have increased greatly. This is in full agreement with mill opinion.

The crop analysis is of value from two points of view. First, the F_2s show that leaf-shape and corolla-colour do not directly affect either yield or the lint characters studied, and it is therefore theoretically possible to breed the desired commercial characteristics into any morphological type. Linkage exists between a factor affecting yield in Malwa and corolla-colour, and between factors affecting halo-length and lint index and leaf-shape.

Secondly among the types actually found in the Malwa crop, the yellow and white corolla components differ markedly in yield and in lint characters. As the F_2s studied were from a cross between two strains unrelated to Malvi cotton (Bani from the Central Provinces and C. 520 from the United Provinces) they give no information concerning the likelihood of the differences observed in the Malvi crop components being linked with corolla-colour. This is a matter which requires investigation. Meanwhile it is clear that the equalisation of genes for yield and lint characters between the morphological types, though theoretically possible, has not in fact occurred. In Malwa this can be satisfactorily accounted for by the low proportion of white corolla types in the crop, and the low percentage of natural crossing. In Nimar, where the main contrasted types—*Malvense* and *Roseum*—are the two largest components of the crop, other factors must be at work, as the low rate of natural crossing alone would not appear to be adequate.

It is now possible to re-state the problem before the plant-breeder. In Malwa he has, not to regain a lost superiority, but to breed strains which will maintain or improve the existing good quality of the produce of the best lands, and respond better than the existing material to the conditions of the inferior lands which have recently come under the cotton crop. In Nimar he should not be content with making the best of a tract producing inferior cotton by breeding for yield and ginning percentage only. The tract has produced a valuable staple cotton in the past and, with his assistance, should do so again.

The data here presented provide information on problems of survival value and selection pressure. The importance of information concerning the mode of action and the efficiency of natural selection can scarcely be over-estimated, but the value of agricultural crops for such studies appears to have been overlooked. There is at present a considerable range of crops on which genetic studies have been made, and of which large areas are sown with seed untouched by the plant-breeder. Quantitative studies of the distribution of known genes in indigenous cultivations of maize, *juar* (*Sorghum* spp.), cowpeas (*Vigna sinensis*) and cotton offer possibilities of obtaining data on survival value which could only be acquired with extreme difficulty from wild plants or animals.

A point of fundamental importance in the study of the effects of selection in crop plants is that, apart from the activities of plant-breeders, there appears to be no tendency for one type to succeed to the exclusion of all others. This may be partly due to invasion from other areas where the selection balance is different, but the figures given in Tables I and II definitely indicate a selection balance and not a tendency towards the preservation of a single type. This state of affairs is in sharp contrast to the status of wild populations of organisms such as *Drosophila* in which there is a 'wild type', variations from which are almost completely eliminated. Neither does it resemble the condition obtaining in polymorphic animals such as the grouse locusts, *Lebistes*, and the land snails, in which polymorphism is associated with close linkage and the 'universal recessive' type of dominance, and appears to depend for its stability on the selective advantage of heterozygotes over homozygotes [Fisher, 1930].

The characteristic features of the selection balance in cotton in Central India are as follows :—

(1) There is no linkage between the factors concerned, no dominance in leaf-shape, and only partial dominance of yellow over white corolla.

(2) In Malwa and Nemawar, the predominant *desi* type is *Malvense*. *Verum* occurs as about twenty-five per cent of the *desi* component, a considerable proportion being heterozygotes for leaf-shape. The white-flowered types are rare, and are apparently kept so by their yield disadvantage. Heterozygous *Verum* does not appear to have any selective advantage over homozygous *Verum* or *Malvense*.

(3) In Nimar, the proportion of yellow-flowered plants is much lower, but the ratio of *Verum* to *Malvense* is about the same. The percentage of *Roseum* is very much greater, but the percentage of *Cutchicum* is not proportionately greater than in Malwa.

(4) In all areas studied, the proportion of *Malvense*+*Roseum* in the *desi* component is much greater than it would be if random assortment occurred between leaf-shape and corolla-colour.

(5) The introduction of Upland (*G. hirsutum*) has added a fresh factor to the situation in the last fifty years. Upland appears to have become established as a permanent component of the system, and occurs in proportions which differ in the different tracts.

No detailed discussion of the incidence of selective forces will be possible until further data are available on the relative yields of different types, and on competition effects in controlled experiments. Meanwhile, however, the nature of some of the more important selection factors can be inferred, and their effect outlined, especially with regard to the balance between Upland and *desi* types.

In the absence of controlled plant-breeding work, human preferences will affect the crop composition in very much the same way as do the forces of natural selection. Four main selective forces will be considered : (1) Yield ; (2) Germination percentage ; (3) Ginning percentage ; and (4) Fineness. Of these, the first two may be classed as natural, and the last two as human selective forces. In the competition between Upland and *desi*, *desi* gains heavily on yield, but loses on germination percentage. In Malwa, Upland gains heavily on fineness, and there is no important difference in ginning percentage. In Nimar, Upland again scores on fineness, but the coarse *Roseum* component gains heavily on ginning percentage. There is no crossing between the Upland and *desi* species, and little crossing between the components of the *desi*, and at least some of the favoured characters are to a considerable extent mutually exclusive (*e.g.*, fineness and ginning percentage). The development of an 'ideal' type is therefore impossible and the selection process results in a balanced mixture. In Malwa, the pressure of the mill interests and the high germinating capacity of Upland seed have resulted in a mixture containing about sixty per cent of Upland. At this point its yield disadvantage is such that further increase is not possible. Increase in ginning percentage by an increase in the proportion of *Roseum* has failed on account of its heavy yield disadvantage, particularly in seasons when frost occurs, but that the selection pressure is there is shown by the periodic introduction of *Roseum* by gin owners. Comparison of the ginning percentages recorded by Hamilton [1849] with those of the present day show that the selection pressure for high ginning has had very little effect. In Nimar, Upland does not thrive so well, though, as in Malwa, there are small areas where it is grown pure. In the greater part of the tract a pure crop of *Malan* is a hopeless failure. In a mixture, on the contrary, it does very well. The conflict of interest is here very well shown by the comparison of crops from ginnery seed and from cultivators' seed. To the cultivator, yield is paramount, and ginning percentage second in importance. The ginners in Nimar find that the extra softness of a mixture in which there is a considerable proportion of *Malan* helps considerably in the sale of the lint and they therefore favour it. The results of selection for high ginning have here been considerable, as Abbott [Return, 1847] records twenty-five per cent for the common cotton, as against twenty-eight to thirty-five at the present day.

This outline of some of the main selective forces at work raises the question whether it will ever be possible to breed a pure type which will be as profitable as a mixture of selected types. No satisfactory answer can be given until information is available on more elusive selection problems, such as competition effects, and the effects of seasonal variation. With regard to the former, some evidence has already been obtained which shows that yield per plant is by no means always the same in a mixture as it is in a pure crop. With regard to seasonal variation, it is of interest to consider the behaviour of Verum 262 cotton in Nimar in the light of the census results. The selective forces affecting quality and ginning percentage will have a similar incidence on *Verum* and *Malvense* types. *Verum* forms about eight per cent and *Malvense* nineteen per cent of the *desi* component. It may therefore be concluded that in the Nimar tract of Central India, *Malvense* is at a considerable selective advantage over *Verum.* Hutchinson and Panse [1936] have published data from seven variety trials in the States areas of Nimar in which Verum 262 was included. Its yields expressed as a percentage of the yield of the local are given below, and for comparison are given the yields of Malvi 9 (a *Malvense* type selected at Indore) for the five trials in which it was included.

Yields of Verum 262 and Malvi 9 as percentages of yield of local, Nimar tract

	Verum 262	Malvi 9
	66	..
	114	119
	112	101
	89	..
	74	98
	79	107
	106	111
Mean	91·4	107·2

The range in yield of Verum 262 is from 66 to 114 per cent, or 48. In Malvi 9, also a strain bred outside the area, and therefore equally likely to be ill-adapted to the vagaries of the Nimar climate, the range is only from 98 to 119, or 21 — less than half that for Verum 262. This suggests that an important reason for the small proportion of *Verum* in the Nimar crop is failure to respond as well as *Malvense* to climatic fluctuations.

Whatever may be the reason for its comparative scarcity it is clear that the census provides extremely valuable guidance to the plant-breeder. Of the types at his disposal, *Roseum* is ruled out on account of its coarseness. Between *Malvense* and *Verum* the choice is clear. The type having a natural advantage is much more promising material than that which occurs as a relatively small fraction of the unselected crop.

One further observation has been made which bears on the problem of the effects of selection. In spite of the very powerful selection in favour of coarse, high-ginning types in the past fifty years, fine cottons persist, and are still to be found in the mixture. The old *M'hahlie* has disappeared, but we have given reasons for believing that it was a type of *G. herbaceum*, and its complete disappearance from the *G. arboreum* var. *neglectum* mixture is not surprising. Fine cottons of the old *Berari* type, however, can still be obtained fairly easily, and it does not seem likely that they would ever disappear entirely under the selection conditions which have obtained recently. It has been pointed out elsewhere [Hutchinson, in press] that in this respect the present coarse staple cotton tracts of the Central Provinces, Berar, Khandesh and the Nimar districts of Central India differ from the coarse *Mattheo* tract of Kathiawar, and the Bengals tract of the Punjab and parts of the United Provinces. In the Central India areas the old fine types that have been superseded persist as more or less rare constituents of the crop, and are available to plant-breeders. In the other tracts, the present crop is the result of an invasion of coarse-stapled types where little or no fine cotton existed before, and the scope for selection is consequently very much less.

VI. Summary

The past history and present status of the cotton crop in Central India has been investigated in an attempt to discover the causes and estimate the extent of the deterioration which it is believed to have suffered. As opportunity offered, information on the Rajputana crop was also collected, and is discussed.

It is shown that the opinions of the local industry and the theories offered by agricultural authorities are not supported by the facts.

The cotton of Malwa, concerning which complaints have been loudest, has suffered no more deterioration than can be accounted for by its spread to less suitable land consequent upon an enormous increase in the area cropped with it. Invasion of *Roseum*, which was supposed to have caused the deterioration, has in fact occurred to only a very small extent, *Roseum* at present constituting only about five per cent of the mixture. The reason for this is that in Malwa it is later and lower in yield than *Malvense*, and is consequently at a heavy selective disadvantage. A very large invasion of Upland has occurred in Malwa, however, and this type, first introduced in 1842, now constitutes about sixty per cent of the mixture. Comparison of the crop composition in villages with a reputation for good cotton with that in other villages in Malwa shows that differences in quality are closely associated with differences in the fertility of the land and, apart from small areas cropped with almost pure Cambodia, do not depend upon the relative proportions of the main morphological types in the mixture.

In Nimar, where deterioration has attracted very much less attention, the quality of the cotton produced a century ago was greatly superior to that of Malwa. Not only was the common country cotton an excellent staple—" not inferior to good Uplands or New Orleans " [Finnie, Return 1847]—but in a small area a particularly high quality cotton, probably belonging to *G. herbaceum*, was produced and exported to Chanderi for use in the manufacture of fine muslins. Invasion by *G. arboreum* var. *neglectum* forma *bengalensis* has taken place in response to the demand for high ginning percentage, and has resulted in deterioration in quality until the crop to-day is markedly inferior to that of Malwa. The old indigenous type has become a comparatively rare component of the mixture, and *Roseum* is now dominant. Upland is grown pure in a small isolated district in Nimar, and exists as a component of the general mixture throughout. In fields grown from ginnery seed it usually comprises thirty to forty per cent of the

crop. Cultivators maintaining their own stocks keep it down below ten per-cent. It is an interesting example of the changes in progress in the cotton trade that ginners now favour Upland at the expense of *Roseum* because of the advantage in marketing.

In Rajputana the crop is of little importance. There are few ginneries and seed stocks are maintained throughout by the cultivators themselves. In consequence of selection exercised according to individual preferences, the crops in neighbouring fields often differ greatly in composition. The *Malvense* type is predominant. Upland has not invaded the area.

A study of yield and quality in F_2s segregating for leaf-shape and corolla-colour showed that there is no necessary genetic association between morphological and agricultural characters, but a factor affecting yield in Malwa exists in the corolla-colour chromosome and factors affecting halo-length and lint index in the leaf-shape chromosome. A similar study of the components of the Malvi crop showed that yellow-flowered types yielded much more than white-flowered, had longer and finer lint, and a lower ginning outturn. It is therefore clear that in the types under investigation there are actually very important associations between agricultural and morphological characters, *Roseum* being coarser, shorter, higher ginning, and, in Malwa, lower yielding than the yellow-flowered types.

The trade evidence for deterioration is discussed. It is concluded that no adequate allowance has been made for short-period seasonal fluctuations. Much of the deterioration also is deterioration in grade and not in the intrinsic quality of the staple. Trade opinions, if carefully analysed, agree on the whole with the results of the crop studies, and show that the best lots of Malvi cotton are as good as ever, while the amount and variability of inferior qualities have increased. In Nimar the occurrence of very serious deterioration is abundantly proved, but most of it seems to have occurred before the local mill industry developed, and is therefore beyond the experience of those from whom opinions were obtained.

The selection balance in the crops of Malwa and Nimar is discussed, and a preliminary outline is given of the effects of some of the more obvious selective forces. It is pointed out that the conflicting nature of these forces, the mutual exclusiveness of some of the characters desired, the low rate of natural crossing between *desi* types and its absence between *desi* and Upland, all contribute to the maintenance of a balance between the crop components. The development of a 'wild type' is the basis, explicit or implied, of almost all discussions of the operation of selection. Even the inadequate discussion of selective forces which is all that is now possible, is sufficient to show that their nature is such as to prevent the evolution of a single, universal type.

VII. Acknowledgments

We have been assisted in the collection of the data here reported by Messrs. Kubersingh, Panse, Simlote, Apte and Govande, of the Institute staff, and we have pleasure in acknowledging our indebtedness to them.

REFERENCES

Bell, H. Hamilton (1843). *Jour. Agric. Hort. Soc., Ind.* **7**, pt. 1, 254.

Calvert, M. and Harland, S. C. (1924). *Jour. Text. Inst.* (*Trans.*) **15**, T8.

Coventry, B. (1916). 'Notes on Tour in Central India'.

Fisher, R. A. (1930). *Amer. Nat.* **64**, 385.

Hamilton, R. C. N. (1849). *Jour. Agric. Hort. Soc., Ind.* **7**, pt. 1, 254.

Howard, A. and Howard, G. L. C. (1929). 'Application of Science to Crop Production' p. 22. Oxford Univ. Press.

Hutchinson, J. B. (1931). *Jour. Genet.* **24**, 325.

———————— (1934). *Ibid.* **28**, 437.

———————— (in press). 'The Distribution of *Gossypium* and the Evolution of the Commercial Cottons'.

Hutchinson, J. B. and Ghose, R. L. M. (in press). "Classification of the Cottons of Asia and Africa."

Hutchinson, J. B. and Panse, V. G. (1936). *Agric. & Livestock Ind.* **6**, 397.

Indian Cotton Committee (1919). *Report ; Govt. of India.*

————————(1920). *Minutes of Evidence, Vols.* **1-6** ; *Govt. of India.*

Return : *Cotton* (*India*) (1847). Ordered by the House of Commons to be printed.

Watt, G. (1890). 'The Dictionary of Economic Products of India', Vol. **4**, p. 97.

Yates, F. (1933). *Emp. Jour. Expt. Agric.* **1**, 129.

VARIATION IN THE CHARACTERS OF COTTON IN RELATION TO THE POSITION OF BOLLS ON THE PLANT

BY

K. R. SEN, D.Sc.

AND

MOHAMMAD AFZAL, B.Sc. (Agri.), A.I.C.T.A.

Cotton Research Laboratory, Punjab Agricultural College, Lyallpur

(Received for publication on 10th October 1936)

(With one text-figure)

Introduction

Several workers have already studied variations in lint-length with respect to the situation of bolls on the plant. For example, Armstrong and Albert [1930] found no apparent correlation between fibre-length and the position of bolls on the plant. Armstrong and Bennett [1933] concluded from their experiments that ' the position of the boll along the vertical axis or the horizontal axis does not seem to be of very great importance in determining the length of the lint '. Kearney and Harrison [1924] had already observed that the differences in mean fibre-length 'as between any two successive groups of fruiting branches ' were not significant in any of the cases studied by them. They mention, however, that on the whole 'bolls from the bottom crop produce shorter fibres than bolls from the top '. It is intended in the present paper to extend the work on the effect of position of bolls so far done to the other important characters of cotton. For, it is well known that the forces of environment acting at any particular time during the period of development of a boll, are not responsible for variation in all the different fibre properties. For example, changes in length do not occur at the same time as those in wall-thickness, as elongation and wall-thickness occur successively rather than concurrently.

The following measurable characters of cotton have therefore been considered here in addition to fibre-length :—

(*i*) Seed and lint indices,

(*ii*) Mean fibre-mass per unit length, and

(*iii*) Mean proportion of mature fibres.

Further, the present experiments were conducted in such a manner that the extent to which the effect of climate supervened that of the position of bolls on the plant could also be studied.

EXPERIMENTAL DETAILS

(a) *Material*

The material utilised for the present experiments was derived from the Punjab-American strain, 45 F, grown at the Cotton Research Station, Lyallpur, during the season 1933-34. This is one of the new early strains with very low sympodial habit and long sympodia.

Ten average plants were labelled on the 7th August 1933. These plants were selected at random from the plot taking care not to include any border plant or any plant showing pre-disposition to disease. It may be stated here that all the labelled plants remained quite normal till the end of the experiment. On each plant ten branches occurring at the following nodes of the main stem along the direction of growth, were marked :—

(1) Bottom zone—Nodes : 4, 5, 6, 7.
(2) Middle zone—Nodes : 22, 23, 24.
(3) Top zone—Nodes : 30,31, 32.

A diagrammatic sketch of the cotton plant is given in Fig. 1.

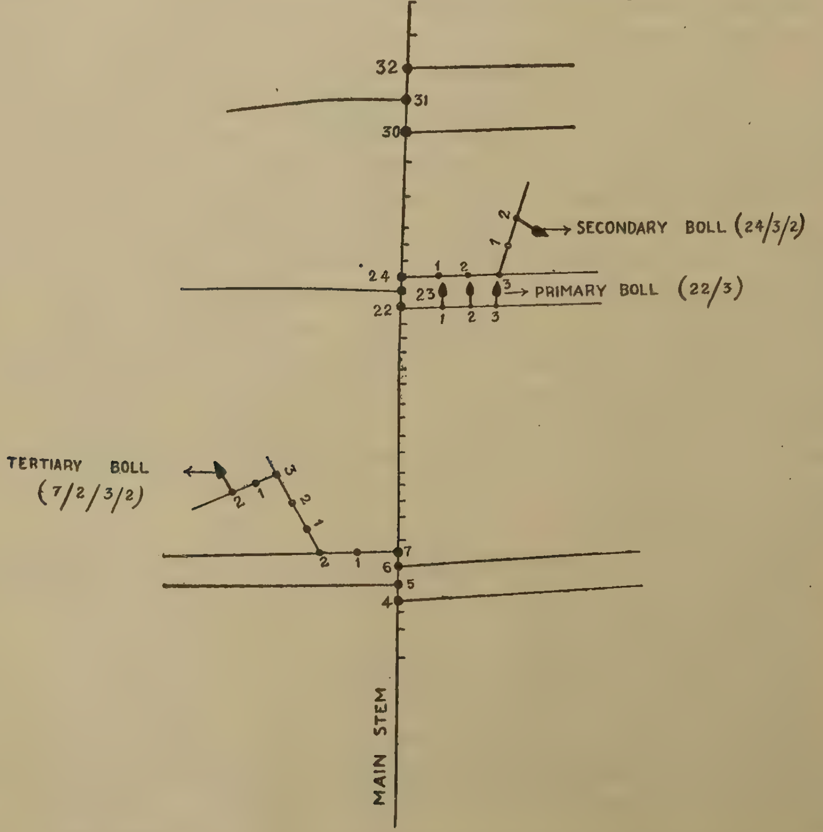


Fig. 1. The position of the primary, secondary and tertiary bolls on the cotton plant.

The flowers which opened on these branches were labelled every morning beginning from the 7th August to 29th September 1933. On the whole 280 flowers were labelled out of which only ninety-three matured into open bolls.

(*b*) *Samples and methods of testing*

The total number of test samples were drawn from forty-three bolls only. With respect to the rest of the bolls, some were 'badly' opened, in some, the fibres were stained, or matted as a result of attack by *Earias* while the remainder were incapable of being classified under the rule adopted for control of maturation period. This rule was that the bolls from flowers labelled on the same day and opening within the successive five-day intervals were lumped together and were considered as having opened on the same day. The fixation of five-day interval was purely arbitrary and was dictated by convenience of sampling and has no biological basis.

The seed and lint indices were obtained by direct weighment on a physical balance. The average fibre-length was determined by Balls' sledge sorter. The mean fibre-mass per unit length was obtained by weighing eight to ten bundles of whole fibres—about 150 to 200 in number in each bundle—on a quartz micro-balance of sensitivity=0·036 mg. per division. The ripe and immature fibres were counted under a microscope after irrigation with 18 per cent caustic soda solution [Clegg, 1930].

The sliver in the case of each sample under test was prepared by picking some fibres at random from eight to ten different parts of the hand-separated lint. These fibres were then subjected to repeated drawing and doubling for a considerable number of times before the draw-box was finally used.

(*c*) *Field observations*

Some plants were observed to produce flowers in abundance in the bottom zone while there were others which produced very few flowers in this zone. On the other hand some plants gave a considerably larger number of flowers in the middle zone than others. The number of flowers in the top zone was always comparatively small. None of the plants labelled for experiment bore any flower on the fifth branch.

As previously observed by several other workers, a critical age was found to exist for the bolls within which the shedding rate was rather high. This age was eight days after flower opening. For bolls surviving this age the probability of shedding was estimated as only nine per cent.

Shedding of bolls in the present case was observed to be about fifty per cent in the top zone, seventy-five per cent in the middle zone and sixty-five per cent in the bottom zone. On the average total shedding was about sixty-seven per cent of the labelled material. Generally, the bolls on the secondary or the tertiary sympodia were mostly shed. On the primary sympodia it was found that a boll near the main stem might frequently be shed while one farther out might stand.

Generally speaking maturation period did not appear to be influenced to any noticeable extent directly by the position of the boll on the plant. With the advance of the season maturation period increased slowly at the beginning and appeared to be comparatively more rapid later on [Loomis, 1927 ; Sen, 1934].

EXPERIMENTAL RESULTS

1. *Primary bolls*

(*i*) *Variation with the position on a branch.*—Only the top zone (beginning with the main stem node 30), afforded samples which could be usefully tested for variations of this type. The results are stated in Table I. For any particular series under examination only bolls maturing within a period of five days of the earliest one, were tested.

TABLE I

Variation along a branch

Serial No.	Boll No.*	Maturation period (days)	Seed-index (grms.)	Lint-index (grms.)	Average fibre-length (cms.)	Fibre-maturity		Mean fibre-mass per cm. at 70 per cent R. H. $(\times 10^{-6})$ grm.
						Ripe (per cent)	Dead (per cent)	
I	30/2	37	7·9	4·6	2·57	81	5	1·74
	30/3	38	8·0	3·1	2·64	45	43	1·35
	30/4	37	8·2	3·3	2·64	38	49	1·30
	30/5	37	7·6	3·5	2·69	52	31	1·42
II	31/1	39	9·8	3·5	2·69	56	30	1·57
	31/2	38	9·6	3·5	2·79	40	45	1·47
	31/3	39	8·3	2·7	2·69	30	50	1·34
	31/4	36	8·0	3·7	2·62	53	22	1·63
	31/5	40	9·4	3·7	2·72	69	16	1·59
III	31/2(*)	43	11·8	3·1	2·67	47	39	1·41
	31/3	42	7·6	3·3	2·54	54	34	1·39
	31/4	41	7·9	3·9	2·64	69	21	1·62
	31/5	41	6·7	2·6	2·49	47	44	1·19
IV	32/1	42	9·8	3·9	2·54	81	9	1·65
	32/2	41	7·5	3·6	2·41	80	7	1·81
	32/3	42	11·0	5·0	2·79	83	10	1·81
	32/4	44	6·7	3·4	2·46	66	15	1·49
	32/5	44	6·9	2·9	2·67	34	48	1·31

*The numerator refers to the branch and the denominator to the position of the boll on the branch.

(*) Some plants gave successive bolls on a branch 31, with maturation period greater than five days with respect to the earliest boll in series II. These are included in series III. The bolls in series III did not differ within themselves by more than five days.

Statistical analysis of these results shows that the fluctuations (as indicated by the coefficient of variability) between positions on a branch become more pronounced when the maturation period is higher, or in other words, the season is more advanced (Table II).

TABLE II

Statistical analysis for primary bolls along a branch

Character of fibres studied	Maturation period 36 to 40 days						Maturation period 41 to 45 days					
	Branch 30			Branch 31			Branch 31			Branch 32		
	Mean value	S. E. (±)	C of V per cent	Mean value	S. E. (±)	C of V per cent	Mean value	S. E. (±)	C of V per cent	Mean value	S. E. (±)	C of V per cent
Seed-index	7·9 grms.	0·22	2·8	9·0 grms.	0·73	8·1	8·5 grms.	1·96	23·1	8·4 grms.	1·72	20·4
Lint-index . . .	3·6 grms.	0·58	16·2	3·4 grms.	0·24	7·1	3·2 grms.	0·47	14·6	3·8 grms.	0·70	18·5
Average fibre-length .	2·64 cms.	0·043	1·7	2·70 cms.	0·055	2·1	2·59 cms.	0·146	5·7	2·57 cms.	0·312	12·2
Mean mass per cm. at 70 per cent R. H. × 10^6.	1·45 grms.	0·172	11·8	1·52 grms.	0·105	6·9	1·40 grms.	0·153	10·9	1·61 grms.	0·310	19·3
$\frac{\text{Dead per cent}}{\text{Ripe per cent}}$ . .	0·71	0·462	65·2	0·80	0·527	66·4	0·67	0·487	72·6	0·39	0·516	132·0

It is easily observable that the ratio of dead to ripe fibres has got the greatest variability. It is also seen that the variability in the case of average length is always lower than that in the case of fibre-mass per unit length.

(*ii*) *Variation along the main stem.*—The mean variation from branch to branch was studied for the following branches: 7, 22, 23, 24, 30 and 32. The results appear in Table III. In this experiment all the bolls on a particular branch constituted the sample for that branch. For example, the values for node 23 is the mean for all bolls on branch 23. This was done because bolls in corresponding positions on different branches—although this would be ideal—were not always available for making up a suitable sample.

TABLE III

Variation along main stem

Node No.	Mean maturation period (days)	Seed-index (grm.)	Lint-index (grm.)	Average fibre-length (cm.)	Mean fibre-mass per cm. at 70 per cent R. H. $(\times 10^{-6})$ grm.	Maturity proportions		Dates of labelling of flowers
						Ripe per cent	Dead per cent	
7	43·5	9·1	4·3	2·63	1·83	71	19	3/9 to 9/9.
22	43·0	6·0	2·5	2·87	1·01	15	78	9/9 (one boll).
23	47·0	6·7	2·6	2·84	1·32	33	50	2/9 (one boll).
24	38·5	6·7	2·9	2·51	1·58	66	18	20/8 to 26/8.
24 (H)*	42·0	5·6	2·5	2·48	1·32	40	47	7/9 (one boll).
30	44·0	6·3	3·1	2·59	1·52	65	26	7/9 to 10/9.
30 (H)	46·5	7·7	3·8	2·52	1·78	74	18	18/8 to 11/9.
32	38·5	7·6	3·2	2·65	1·46	58	28	26/8 to 28/8.

* Those marked (H) refer to higher maturation period for the same branch of different plants according to the adopted rule of five-day limit.

It can be gathered from the statistical analysis of these data given in Table IV that in the case of the present experiments fluctuations between branches seem to be in some cases higher than those between positions along a branch.

TABLE IV

Statistical analysis of variations from branch to branch

	Seed-index	Lint-index	Average fibre-length	Mean fibre-mass per unit length at 70 per cent R. H.	$\frac{\text{Dead per cent}}{\text{Ripe per cent}}$	Remarks
Mean value . . .	6·7 grms.	3·1 grms.	2·64 cms.	$1 \cdot 48 \times 10^{-6}$ grm.	1·17	
S. D.	±1·06	±0·61	±0·139	±0·250	±1·849	
C of V (per cent) . .	15·1	19·4	5·3	16·9	...	Coefficient of variation is so high that the mean value itself judged by the S. E., is non-significant.

With regard to the effect of different maturation periods on the mean values, it can be seen from Table V that the variations, however apparent, are not statistically significant.

TABLE V

Statistical significance of the difference between two mean values corresponding to different maturation periods

Characters tested	Branch No. 31	Maturation periods : 36 days and 41 days			
	Difference between mean values	S. E. E_{36} for maturation period of 36 days (±)	S. E. E_{41} for maturation period of 41 days (±)	2 (S. E. of diff.) or $2\sqrt{E_{36}^2+E_{41}^2}$ (±)	Remarks
Seed-index	0·50 grm.	0·3640	1·1290	2·37	Not significant.
Lint-index	0·20 grm.	0·1202	0·2690	0·59	Do.
Average fibre-length . .	0·115 cm.	0·05495	0·08420	0·201	Do.
Mean fibre-mass per cm. at 70 per cent R. H. × 10^6.	0·12 grm.	0·1044	0·0880	0·273	Do.
$\frac{\text{Dead per cent}}{\text{Ripe per cent}}$ . . .	0·13	0·5273	0·2810	1·18	Do.

Analysis of variance, by Fisher's method [1934] of the data for primary bolls given in Tables I and III, shows that in spite of all apparent fluctuations in the values neither the position of the boll on a branch, nor the position of a branch on the plant, nor in fact the limit of differences prescribed for the maturation period, has any significant effect (Table VI) on length, mass or maturity of fibres. The apparent fluctuations can only therefore be due to any irregular fluctuations of biological origin co-existent with fluctuations due to climatic [Sen, 1934, 1] and water [Sen, 1934, 2] conditions.

TABLE VI*

Values of 'z' from Fisher's table for studying significance

n_1	n_2	'z' for $P=0·05$	'z' for $P=0·01$
3	3	1·1137	1·6915
1	3	1·1577	1·7649

Data analysed to study the effect of	Variance due to	Degrees of freedom	Mean sum of squares					
			Maturation period 36 to 40 days			Maturation period 41 to 45 days		
			Average length	Mean mass ÷ length at 70 per cent R. H.	$\frac{\text{Dead per cent}}{\text{Ripe per cent}}$	Average length	Mean mass ÷ length at 70 per cent R. H.	$\frac{\text{Dead per cent}}{\text{Ripe per cent}}$
Position on branch.	Position .	3	0·00197	0·02313	0·3275	0·00645	0·05838 (z=0·2221)	0·00949
	Branch .	1	0·00980 (z=0·1953)	0·00605	0·0444	0·00003	0·08202 (z=0·3919)	0·08715
	Remainder	3	0·00543	0·03312	0·3930	0·03527	0·03744	0·48112
			For branch No. 31 only					
Different maturation periods for the same branch.	Position .	3	0·00693 (z=0·1218)	0·02763 (z=0·1529)	0·26105 (z=0·0134)			
	Maturation period.	1	0·02880 (z=0·8342)	0·02205 (z=0·0403)	0·07088			
	Remainder	3	0·00543	0·02035	0·25416			

---

*In this table the values of 'z' have been calculated only where the 'Mean sum of squares' for the cause of variance concerned is greater than that for the 'remainder'. Only such cases have the likelihood of being significant in the present experiments. The calculated values of 'z' are placed below the corresponding 'Mean sum of square' within brackets.

2. *Secondary and tertiary bolls*

So far as the secondary bolls are concerned the first bolls on the secondary sympodia growing at the successive nodes 8, 9, and 10, on the particular branch (No. 23) were available for testing. These bolls had the same maturation period. The results of tests for these bolls are given in Table VII. In the same table also occur the results for the only two available tertiary bolls which also had the same maturation periods.

The results obtained by testing either the secondary or the tertiary bolls, due to their small number, cannot be regarded as definite. So the conclusions should be considered as merely tentative.

TABLE VII

Results for the secondary and the tertiary bolls

Class of bolls	Boll No.*	Maturation period (days)	Seed-index (grms.)	Lint-index (grms.)	Average fibre-length (cm.)	Maturity proportions (per cent)		Mean fibre-mass per cm. (grm. × 10^{-6}) at 70 per cent R. H.	Remarks
						Ripe	Dead		
A. Secondary	23/8/1	38	6·4	3·3	2·50	53	29	...	* References :— 1st figure—Main node. 2nd figure—Secondary sympodial node. 3rd figure—position of boll. *e.g.*, 23/8/1 refers to the 1st boll on the 8th secondary sympodial branch of the 23rd main stem branch.
	23/9/1	39	8·6	4·1	2·58	64	20	...	
	23/10/1	40	8·9	3·1	2·57	77	11	...	
B. Tertiary	6/16/3/2	48	6·9	2·7	2·49	42	45	1·28	* References :— 1st figure—Main node. 2nd figure—Secondary branch No. 3rd figure—Tertiary sympodial branch No. 4th figure—Position of the boll.
	22/4/2/1	50	8·3	3·6	2·49	70	24	1·80	

With respect to the secondary bolls, it seems that the boll nearest the main stem gives inferior fibres to those farther out. The only features that can be remarked upon in the case of the two tertiary bolls are :—

(*i*) The average fibre-length, as tested by the method of standard error of the difference, does not vary significantly.

(*ii*) The ratio of percentages of dead to ripe fibres in the case of the lower boll is overwhelmingly greater than that in the other case.

Both these tertiary bolls possessed high maturation period compared with the primary or the secondary bolls studied.

3. *Primary bolls irrespective of branch*

In addition to all the above series, another one consisting of primary bolls irrespective of the branch on which they grew, but possessing the same maturation period, were tested. It may be stated that the ripening of these bolls followed the same order as in labelling. The results are given in Table VIII.

Table VIII

Result for bolls labelled on successive days

Boll No.	Plant No.	Maturation period (days)	Date of labelling of flower	Seed-index (grms.)	Lint-index (grms.)	Average fibre-length (cm.)	Maturity proportion (per cent)	
							Ripe	Dead
31/4	III	41	24/8/33	9·6	3·1	2·69	50	22
32/3	VI	39	27/8/33	7·6	3·9	2·57	60	21
32/3	X	39	28/8/33	8·7	3·6	2·57	59	20
30/3	IX	39	29/8/33	9·8	4·2	2·62	80	8
31/4	X	39	31/8/33	8·6	3·5	2·50	47	37

The number of bolls for this experiment was only five and no definite conclusions can be drawn. It may, however, be mentioned that variations in the different characters are generally very small, although with respect to average length and maturity certain bolls show marked difference from others. It may, therefore, be said that variation in fibre properties depends mainly on environmental factors and are independent of the position of the boll on the plant.

Discussion

The very fact that the variations in fibre characters with reference to the position of the boll on the plant are found to be statistically insignificant, does not altogether preclude the possibility of some variations really occurring. It only shows that the apparent resultant effect of the diverse conditions is not considerable. Moreover, the nature of the experiment itself involves an inherent difficulty in the way of obtaining suitable series of considerable data. This difficulty is due to shedding of labelled bolls, of which the remedy is beyond control at the present state of our knowledge. Accidental breaking off of bolls from their attachments on the branches on account of wind, etc., also contribute their quota in this respect. All these factors make it imperative that all available data should be analytically studied before any conclusion is definitely drawn.

The fundamental factors which produce any impression on cotton fibres as regards their physical quality, are moisture and temperature, provided, as Massey [1915] states, other cultural conditions are not abnormal. Even manure, or in other words, the chemical composition of the soil which so obviously affects the yield of seed-cotton per acre [Bartholomew and Nansen, 1928 ; Naftel, 1931 ; Armstrong and Bennett, 1933] seems to produce no significant effect on fibre characters. The chemical constitution of the soil, as is now known from the works of the foregoing authors, acts on the fibre characters only in so far as it affects the moisture-retaining capacity of the soil. The work done by one of the present writers [Sen, 1934, 2] on manuring experiments at Lyallpur, has also shown that the above conclusions hold not only for fibre-length, but for fibre-maturity and fibre-mass per unit length also.

In order, therefore, to investigate the possible causes of variations in the fibre properties of cotton it is necessary to fix attention primarily on the humidity and temperature conditions during the period of boll development. It is well known that cotton plants carry on growth activities during the night, although photo-synthetic activity of plants rises to a peak during the day time. But very high day temperatures of course are unfavourable to photo-synthesis and in spite of favourable night temperatures, will result in decreased growth activity [Zaitsev, 1925]. Evidently when the day temperatures are fairly uniform, the phenomenon of growth and development should depend more intimately on the night temperature than on the day temperature. Now, in case the change both in the day and the night temperatures is uni-directional, it is very easy to study the rising or the falling tendency of the temperatures. Not so however in the present case, as can be seen from Table IX which gives the weekly mean values of these two factors of environment. In order, therefore, to find a common basis for judging the general tendency to change, of both day and night temperatures together, the following criterion has been adopted. The increase in the ratio of the difference between the maximum and minimum temperatures to the mean temperature, will indicate the relative fall in the night temperature when the day temperature is fairly uniform, as in the present case up to 16th October 1933. Thus this ratio is a suitable criterion which will determine whether the temperature conditions are favourable or not. These figures are also given in Table IX. From 17th October 1933 both day and night temperatures fell quite markedly.

As regards humidity it is realised that relative humidity at 8-0 A.M. cannot always truly indicate the humidities prevailing during the twenty-four hours of the day. The 8-0 A.M. values indicate, however, in a broad manner the progress of the climate from wet to dry conditions. Moreover, 8-0 A.M. readings of relative humidity constitute the only available record.

TABLE IX

Weekly mean values of environmental factors

Labelling of flowers commenced on 7th August 1933, collection of bolls stopped on 20th November 1933.

Period (year 1933)		Mean weekly air temperatures (°F.)		Difference in temperature (°F.)	Mean temperature (°F.)	Temperature difference / Mean temperature	Mean weekly R. H. at 8-0 A.M. (per cent)	Remarks
From	To	Maximum	Minimum					
1/8	7/8	94·2	78·8	15·4	86·5	0·178	75·3	
8/8	14/8	93·9	80·3	13·6	87·1	0·156*	80·4	*Indicates fluctuations due to rainfall in the temperature values.
15/8	21/8	94·0	78·4	15·6	86·2	0·181	80·3	
22/8	28/8	86·9	76·9	10·0	81·9	0·122*	85·4	
29/8	4/9	91·5	75·3	16·2	83·4	0·194	82·7	
5/9	11/9	93·8	77·1	16·7	85·5	0·195	73·3	
12/9	18/9	95·7	75·7	20·0	85·7	0·233	71·4	
19/9	25/9	94·8	74·9	19·9	84·9	0·234	74·3	
26/9	2/10	96·0	72·6	23·4	84·3	0·278	64·1	
3/10	9/10	98·0	68·3	29·7	83·2	0·357	50·1	
10/10	16/10	93·9	67·0	26·9	80·5	0·334*	51·4	
17/10	23/10	90·1	60·7	29·4	75·4	0·390	60·1	
24/10	30/10	89·5	56·9	32·6	73·2	0·445	47·6	
31/10	6/11	88·5	53·6	34·9	71·1	0·491	53·9	
7/11	13/11	85·7	50·6	35·1	68·2	0·515	52·6	
14/11	20/11	80·5	52·4	28·1	66·5	0·423*	65·9	

It will be seen from Table IX that the temperature, in spite of slight fluctuations on account of rainfall, generally fell as the season advanced, as indicated by the above-mentioned ratio. The relative humidity also appears to have fallen almost continually since the end of August.

The flower and boll production in cotton continues in a spiral which gradually covers the outer regions along the branches and the upper and the lower regions along the main stem. The branch on which the spiral of flowering should commence, is determined, apart from genetic factors, by the temperature and moisture conditions [Zaitsev, 1927]. It is clear, therefore, that the farther a boll is from the main stem along a branch, the later will it set and develop. This also holds good as we proceed up or down the main stem from the centre of flowering. Consequently, if there is progressive change in either the temperature or humidity, it can be expected that the fibres will deteriorate or improve depending upon the nature and extent of fluctuations of the limiting factor. It was shown by one of the present writers [Sen, 1934, 1] that low temperature as well as high humidity are the factors which contribute to the unfavourable environment for the cotton fibres. In the present case as the season advanced into and beyond September, the humidity showed progressive tendency to fall, but the temperature showed fluctuations apparently superimposed on a general tendency towards a fall. In spite of falling humidity, therefore, the temperature was the more important limiting factor in the present case. The detrimental influence of falling temperature is therefore expected to be somewhat moderated by the favourable effect of falling humidity. Further, any depressing effect which this change of temperature may have entailed, might possibly have been considerably aggravated by the approaching senility of the plant. Under such circumstances, on a general tendency towards deterioration of fibre quality (as the outer bolls on a branch or the upper or lower bolls along the main stem are encountered) will be superimposed the consequent fluctuating changes on account of any irregular alterations in the temperature. Fluctuations in humidity should also prove to be an active factor in modifying the general effect of falling temperature. Thus, if temperature rises while humidity falls, the effect of climate will be to improve fibre properties ; conversely, deterioration in the fibre characters will take place when the conditions of temperature and humidity are reversed. If however one of these two factors changes in the favourable direction, while the other changes in the unfavourable direction, the deterioration on account of the one may be checked, and even lessened, by the other. So the resultant effect of humidity and temperature on fibre properties is highly complex depending upon the manner in which one changes relatively to the other. From the perusal of the results also it does not appear improbable that some fluctuations, however small, in the fibre characters may have occurred on account of superimposed irregular changes in the climatic conditions.

SUMMARY

(1) Bolls seem to possess a critical age within which the odds are heavily against their living up to maturity.

(2) The percentage of boll-shedding at any time of the flowering period seems to depend almost directly on the mean moisture percentage of the atmosphere.

(3) By analysing the data it is found that fibre properties do not depend on the position of the boll on the plant as such, all observed fluctuations being explicable with reference to the effect of climate.

ACKNOWLEDGMENTS

(1) This work was conducted as a part of the Punjab Botanical Research Scheme of the Indian Central Cotton Committee, financed conjointly with the Punjab Government.

(2) The authors wish to convey their thanks to Mr. B. K. Kar, M.Sc., for many useful suggestions, and to Mr. B. R. Sehgal, M.A., for revision of the statistical work.

REFERENCES

Armstrong, G. M. and Albert, W. B. (1930). *S. Caro. Stn. Rept.* (Abs. from *E. S. R.* **64**, Pp. 628).

Armstrong, G. M. and Bennett, C. C. (1933). *Jour. Agric. Res.* **47**, 7.

Bartholomew, R. P. and Nansen, G. (1928). *Jour. Amer. Soc. Agron.* **20**.

Clegg, G. G. (1930). *E. C. G. C. 1st Conf. Proc.*

Fisher, R. A. (1934). " Statistical Methods for Research Workers ", Oliver and Boyd, London.

Kearney, T. H. and Harrison, G. J. (1924). *Jour. Agric. Res.* **28**, 563.

Loomis, H. F. (1927). *Dept. Agric.* (*U. S.*) *Bull.* No. **1365** (Abs. from *E. S. R.* **57**, Pp. 34).

Massey, R. E. (1915). *Ed. Rep. (Anglo-Egypt Sudan) Bull.* No. **1**.

Naftel, J. A. (1931). *Jour. Amer. Soc. Agron.* **23**, 2.

Sen, K. R. (1934, 1). *Ind. Jour. Agric. Sci.* **4**, 295.

——— (1934, 2). Thesis, Dacca, Univ. (Published by the author).

Zaitsev, G. S. (1925). " The Cotton Plant " Centr. Dep. Industr. Propag. Publi. Moscow.

——— (1927). Trans. Turkest. Plant Breed. Stn., Tashkent, **7**.

STUDIES IN INDIAN BARLEYS

IV. THE INHERITANCE OF SOME ANATOMICAL CHARACTERS RESPONSIBLE FOR LODGING AND OF SOME EAR-HEAD CHARACTERS IN AN INTERSPECIFIC CROSS BETWEEN TWO PUSA BARLEYS *

BY

R. D. BOSE, B.Sc.

Offg. Assistant Economic Botanist, Pusa

M. A. AZIZ, B.Sc. (Agri.)

AND

M. P. BHATNAGAR, B.Sc. (Hons.)

Post-graduate Students in Botany, Pusa

(Received for publication on 6th August 1936)

(With Plates I-III and four text-figures)

I. Introduction

Twenty-four different types of barleys have been evolved and classified at Pusa [Bose, 1931]; these include five types of two-rowed barleys, *Hordeum distichon* L. and nineteen types of six-rowed barleys belonging to the species *Hordeum vulgare* L. var. *Pallidium*. As is well known these two species of the crop can be differentiated from each other by the sterility or the fertility, as the case may be, of the lateral florets.

In India the cultivated form of barley is invariably the six-rowed one. These barleys usually give higher yields of grain than the two-rowed forms, but are generally inferior to the latter in plumpness and in the uniformity of the grain size. Pusa type 21 has proved itself to be a very high-yielding strain with rather stiff and strong straw and hence it is gaining favour as an ideal barley for Bihar conditions. Owing to the presence of a purplish tinge on the seed-coat, type 21, however, has certain limitations as a malting barley, because it is said to impart a rather deep tint to the beer manufactured out of it. This barley is medium early in maturity.

Pusa type 1 is a very early-maturing, two-rowed barley and differs from all other Pusa types in possessing broad outer glumes and very plump grains. The seed colour is light yellow and very attractive. This type however, is an extremely poor yielder and is very susceptible to lodging.

---

*Abridged from the undermentioned papers read before the 22nd Meeting of the Indian Science Congress, Calcutta, 1935 :—

1. Inheritance of some anatomical characters responsible for lodging in barley.
2. Inheritance of some characters in the barley ear-head.

In order to combine the high-yielding quality and the standing power of type 21 with the plumpness and good grain colour of type 1 these two barleys were hybridized. A number of promising hybrids have been secured and are under trial. It is the purpose of this paper to describe the mode of inheritance of (1) some anatomical characters responsible for lodging and (2) some characters of the ear-head, and it is proposed to deal with them in Parts I and II of this paper respectively.

II. MATERIAL AND METHOD

The two types of barley employed for this investigation may be described as follows [Bose, 1931; Bose and Dixit, 1931]:—

Type 1.—Two-rowed. Early, habit erect; root-system mesophytic B, poorly developed; leaf-sheath colour green; foliage light green, short and narrow leaves; tillering poor; height (57 cm.), short; density 32·1; ear short, dense outer glumes broad and awned; kernel hulled; short and plump, seed of light yellow colour; weight of 1,000 grains 45·0 grms., bushel weight 48 lb., maturity early (65 days).

Type 21.—Six-rowed. Early, habit semi-erect; root-system mesophytic, vigorous development; leaf-sheath colour green; foliage bluish green, leaves long and broad; tillering good; height short (83 cm.); density 30·2; ear medium long, broad and rather dense; kernel hulled, long and plump white seed with a purplish tinge; weight of 1,000 grains 38·1 grms., bushel weight 45 lb.; maturity medium early (79 days). Glume and awns purple, fading to lighter shades on ripening. This is the best yielder under North Indian conditions.

Pure lines of the parent types were always grown along with other barleys in fields of average fertility and crossing was done in the field. Emasculation had to be carried out before the ear-heads came out of the leaf-sheaths. In the case of type 1 particularly, fertilisation invariably takes place long before the ear heads out and naturally very young spikes had to be chosen for emasculation and pollination. The cross between type 21♀ × type 1♂ was successful but owing to the lateness of the season when the work was undertaken, the reciprocal cross was not secured. At the suggestion of the senior author the cross was made by Mr. R. G. Joglekar, a post-graduate student in the Botanical Section at Pusa, to whom our sincere thanks are due.

The F_1 plants were grown on rather rich soil and showed some lodging. The F_2 and F_3 generations were raised under the usual field conditions with a spacing of three feet between rows and one foot between plants in the row, in fields of average fertility to which no manures of any kind were added. The F_2 generation from the same seed was raised once in 1932-33 and then again in 1933-34. The F_3 generation was grown only in 1933-34 and consisted of the progenies of a large number of F_2 plants from the previous year.

The material for the anatomical studies was collected after internodal growth had completely ceased but before the grain was actually ripe. A piece of about 3 cm. in length was cut at the third internode from the top, from the main culm of each plant and preserved for a few months in formalin alcohol. Pieces from different F_2 plants were kept separately in sample tubes, while those from each culture in the F_3 generation were strung together. Permanent mounts were made of transverse free-hand sections of the culms from all F_2 plants and the sections were stained with safranin. No permanent mounts were made in F_3.

Part I—The inheritance of some anatomical characters responsible for lodging

That lodging in cereal crops such as wheat and barley is a perplexing problem for plant breeders, is a fact that goes without saying and the attention of many workers has of late been drawn to this very intricate and complex problem. Most of the work on the subject deals with the causes and factors which bring about this phenomenon, a few deal with the relationship of morphological or anatomical characters to lodging and a very few are concerned with the inheritance of the general standing power of the crop, but no work perhaps has till now been published to bring out the nature of the inheritance of anatomical characters which are responsible for lodging in cereals. The present studies furnish evidence that like external morphological characters, certain internal anatomical characters also are inherited on Mendelian principles.

Lodging is the laying over of cereal plants at a period when their vegetative growth is nearly completed and consequently it interferes with the normal development of grain and affects very materially both its quality and the quantity. The accepted opinion is that lodging is generally caused by nitrogenous over-nutrition, as this phenomenon occurs most frequently on naturally fertile soils, rich in humus, or on soils heavily fertilized. The loss due to lodging depends therefore on the degree of falling, the state of development at which it occurs and the subsequent weather conditions.

HISTORICAL

The work hitherto done on lodging may be broadly reviewed under three main heads :—

(1) Observational,
(2) Chemical, and
(3) Anatomical.

We shall briefly discuss each point under which the subject has been dealt with.

(*1*) *Observational.*—It has been observed by many workers that lodging is due to the presence of excessive nitrates in the soil. Thaer [1848], an English physician and agriculturist, was among the earliest to take note of lodging in wheat and attributed the phenomenon to rich ground, abundant manuring, poor ploughing, thick sowing and sometimes to a diseased condition.

Howard and Howard [1912] have laid great stress on the importance of the root-system in wheat and its relation to lodging and recognised that the character for stiff straw must be combined with a strong root-system in a wheat which was to be resistant to lodging and successfully produced a hybrid with these two heritable qualities. They also studied the inheritance of standing power in a cross between two wheats and claim that they got definite differences in stand in the F_2 but did not furnish actual figures or ratios obtained. Nilsson-Ehle, cited by Tschermak [1923], found transgressive segregation when breeding wheat for strong straw. Harlan and Hayes [1919] crossed Manchuria and South African barleys and secured hybrids with stiffer straw and better yields than Manchuria. Schribaux [1920] found greater resistance to lodging and greater strength of straw and yield among the progeny of Ligowo × Brie oats. Hunter's [1919] and Beaven's experiments in England have produced strongly strawed and high-yielding hybrid barleys which are now being cultivated on large areas in that country. Shaw and Bose [1933] have evolved a number of hybrid oats at Pusa from crosses between the Scotch Potato and two Pusa oats and some of these show marked stiffness of straw and high yields. Biffen and Engledow [1926] reviewing the problem note the immense diversity of types of straw among the progeny of the crosses of Rivet with any English wheat.

A number of investigators have studied the breaking strength of straw in relation to lodging. Willis [1925] described an apparatus for such tests. Salmon [1931] found that the soft winter wheats had the highest breaking strengths whereas the hard winter wheats showed the lowest breaking strength. Clark and Wilson [1933] found no correlation between the breaking strength and lodging behaviour.

(2) *Chemical.*—Weakness of straw has been attributed to the lack of various elements, particularly silicon and potassium. That lack of potassium silicate was responsible for lodging was first suggested by Davy [1821]. This view, however, has fallen into disrepute by reason of the fact that plants have been found to make normal growth, even to maturity, in nutrient solutions free from silicic acid, and because much of the silica found in the Gramineae is contained in the leaves rather than in the culms. Another factor which contributes towards the causes of lodging is a deficiency of the lignin content of plants which is believed to lend mechanical support to the stalks. Dadswell and Hawley [1929], on the other hand, consider that a high lignin content is one of the main causes of lodging. Welton [1928] suggested that lodging in wheat and oats was associated with low content of dry matter per unit length of culms and with the etiolation due to shading. He also believed that hypernutrition not only tended to induce a greater elongation of culms, but through a stimulation of tillering process, it increased thickness of stand and thus brought about a greater degree of shading around the base of the plants. In warm, rainy, cloudy seasons, therefore, the conditions were most favourable for the development of lodging on rich soils.

(3) *Anatomical.*—Lienau [1903], Vageler [1906], Albrecht [1908] and others have studied lodging in relation to anatomical structures of the plants but most of these workers failed to find any relationship between the anatomical structures studied and lodging. Garber and Olson [1919] studied the anatomical characters in relation to lodging in oats, wheat and barley and concluded that thinness of cell-walls seemed to be associated with lodging. They believed that lodging was dependent upon so many factors, unequal in value, that no one factor seemed to be correlated closely enough to be of much value as a selection index. Bhide and Bhalerao [1925] in their investigation on rice found that the thicker band of sclerenchyma at the periphery, numerous vascular bundles and the presence of small sub-epidermal girders were absent in the lodging forms of the crop.

Ramiah and Dharmalingam [1934] have quite recently published a paper in which they state that the morphological studies on the rice plant show that there is no single factor which could be used as an index of the lodging nature of straw and from the study of crosses between two typically lodging varieties and a typically non-lodging variety of rice they suggest that there is only a single factor difference between the two. They have also found genetic association between the character of the straw on one hand and tillering and duration on the other. Non-lodging nature of straw is said to be linked up with poorer tillering and longer duration. No relationship has been found between straw character and plant height and straw character and floret sterility.

ANATOMICAL DESCRIPTION

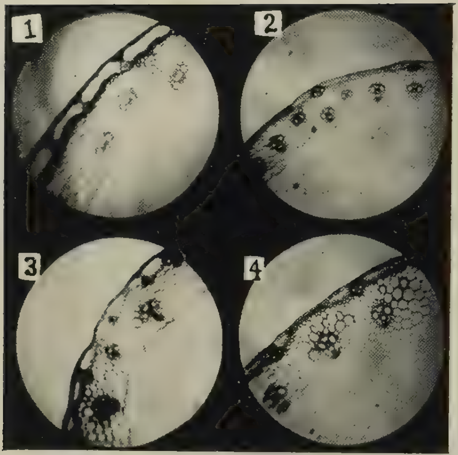
The material used in these studies comprised of F_2 and the F_3 generations of the cross, Pusa type 21 × Pusa type 1 barleys and the two parents. A comparison of the important anatomical characters shown by the F_2 phenotypes is summarized in Table I.

TABLE I

Comparison of anatomical characters studied

Characters	Like type 1	Inter.-A	Inter.-B	Like type 21
1. Arrangement and position of the peripheral sclerenchyma.	Very close to the periphery.	Generally close to the periphery but at places away from it.	Mostly away from the periphery and connected at places by irregularly-placed sub-epidermal girders.	Away from the periphery and connected to it at regular intervals by well-developed sub-epidermal girders.
2. Development and width of peripheral sclerenchyma.	Poorly developed, 9·7 units or 28 μ.	Well developed, 10·7 units or 31 μ.	Well developed, 10·7 units or 31 μ.	Very well developed, 15·1 units or 43 μ.
3. Development and average thickness of sclerenchymatous cell-wall	Poorly developed, 0·9 units or 2·6 μ.	Fairly well developed, 0·9 units or 2·6 μ	Fairly well developed, 0·9 units or 2·6 μ.	Very well developed, 1·1 units or 3·1 μ.
4. Average diameter of the tangential axis of the vascular bundles.	8·7 units or 145 μ.	10·0 units or 167 μ.	10·2 units or 169 μ.	10·8 units or 181 μ.
5. Average diameter of the radial axis of the vascular bundles.	7·6 units or 127 μ.	8·6 units or 144 μ.	8·7 units or 145 μ.	9·9 units or 165 μ.

Units represent the number of divisions on the eye-piece micrometer.



Microphotographs showing transverse sections of barley culms with different types of mechanical tissue.

1. Non-lodging barley like type 21 ; *2.* Lodging barley like type 1 ; *3.* Slightly lodging like Inter-B ; *4.* Almost fully lodging like Inter-A.

It may be noted from the above table that type 1 barley which is very susceptible to lodging has its sclerenchymatous tissue developed very close to the periphery and that the development of the sclerenchymatous tissue and the thickness of its cell-wall is very poor indeed. The size of the vascular bundles too is the least in this type. Type 21, on the other hand, has a very well-developed sclerenchymatous tissue, lying away from the periphery but connected to it at regular intervals by well-developed sub-epidermal girders (Plate I). The size of the vascular bundles too is much greater than that in Type 1. It may be pointed out that the type of mechanical tissue depicted by barley type 21 is very suitable to bear the mechanical strains to which the stem of this plant usually is subjected. In the words of Haberlandt [1914] " This is evidently a particularly effective arrangement, and as a matter of fact analogous types of construction find a wide application in architectural and engineering practice." The development of the mechanical tissue and the size of the vascular bundles in the two intermediate phenotypes are more or less midway between the susceptible and the resistant parents. Inter.-A resembles somewhat type 1 parent while Inter.-B approaches type 21.

All the anatomical characters enumerated above in Table I appear to be very strongly associated and it is presumed that they have a common genetical basis. It is proposed to describe first the individual characters separately and then to study their correlated inheritance. The importance of the latter in these studies can hardly be over-emphasized.

INHERITANCE OF MECHANICAL TISSUE

The arrangement and position of the peripheral sclerenchymatous tissue was found to be the most important character studied. On it depended the susceptibility or resistance to lodging to a very marked degree. Four types of phenotypes were recognised in the F_2 on the basis of this character and the F_3 study fully confirmed this fact. The phenotypes were :—

1. Like type 1.—When the sclerenchymatous tissue was poorly developed and was close to the periphery (Fig. 1-*b*).
2. Inter.-A.—When the sclerenchymatous tissue was developed close to the periphery but at places it was separated from it by ordinary parenchyma (Fig. 1-*d*).
3. Inter.-B.—When the sclerenchymatous tissue was developed away from the periphery and was connected to it at places by irregularly-placed sub-epidermal girders (Fig. 1-*c*).
4. Like type 21.—When the sclerenchymatous tissue was well developed and was away from the periphery and connected to it, at regular intervals, by well-developed sub-epidermal girders (Fig. 1-*a*.)

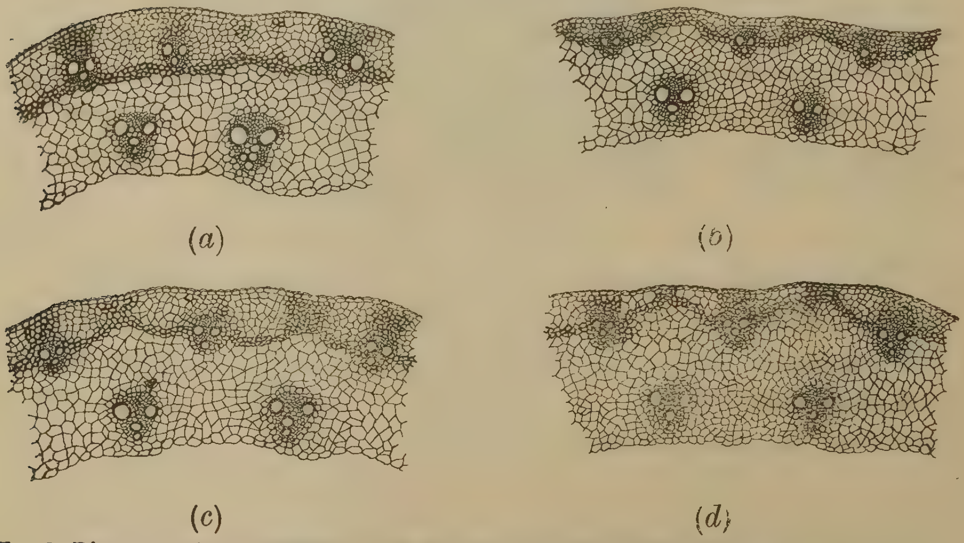


Fig. 1. Diagrammatic representation of the four phenotypes of mechanical tissue found in transverse sections of the Barley culm.

(*a*) Non-lodging barley like type 21 ; (*b*) Lodging type like type 1 ;
(*c*) Inter.—B ; (*d*) Inter.—A barleys.

It may be pointed out that the strain or impact of wind or any mechanical force is first borne in a non-lodging form like type 21 by the regularly-placed sub-epidermal girders which greatly strengthen the mechanical tissue, whereas in a plant which is susceptible to lodging the whole strain falls directly over the imperfectly-developed sclerenchymatous band.

In the F_2 the following phenotypes and frequencies were observed :—

TABLE II

F_2 segregation for the inheritance of mechanical tissue in relation to lodging in barley type 21×type 1

Year	Frequency	Mechanical tissue like				Total number of plants
		Type 1	Inter-A	Inter-B	Type 21	
1932-33 . .	Observed*	151	53	52	15	271
	Expected	152·46	50·82	50·82	16·94	
1933-34 .	Observed	101	36	34	13	184
	Expected	103·50	34·50	34·50	11·50	
Total . .	Observed	252	89	86	28	455
	Expected	255·96	85·32	85·32	28·44	

$\chi^2=0{\cdot}2318$, *P* between 0·95 and 0·98. The fit is excellent.

*The observed frequencies were slightly modified and corrected after the study of the F_3

Both these F_2 generations were grown from the seed of the same F_1 plant and were identical in behaviour. It seems quite safe, therefore, to conclude that the inheritance of mechanical tissue depends on the interaction of at least two genetic factors giving a ratio of 9 : 3 : 3 : 1 with plants possessing mechanical tissues like type 21 forming the double recessive class.

The F_3 data shown in the following table give good substantiation of the hypothesis based on F_2 results.

TABLE III

F_3 behaviour for the inheritance of mechanical tissue

Nature of F_2 parent	No. of families	F_3 behaviour	No. of families	
			Observed	Expected
Like type 1 . . .	129	Type 1 pure	11	14·25
		Type 1 : Inter.-A	27	28·50
		Type 1 : Inter.-B	28	28·50
		Like F_2	63	57·00
Inter.-A	43	Inter.-A pure	14	14·25
		Inter.-A : Type 21	29	28·50
Inter.-B	45	Inter -B pure	15	14·25
		Inter.-B : Type 21	30	28·50
Like type 21 . . .	11	Type 21 pure	11	14·25

The data for the observed and expected families are in harmony.

INHERITENCE OF THE DEVELOPMENT OF THE SCLERENCHYMATOUS TISSUE

In a recently published paper Brady [1934] has shown that the cell-wall measurement of the sclerenchyma in the samples of oat varieties studied by him was too unstable a character to be of any use as an absolute criterion of the lodging-resistant ability of a variety and that the most that could be expected from the characters examined would be their possible value as relative indicators of resistance to lodging. He thinks that in this capacity their usefulness would depend on the uniformity of the environmental conditions under which the varieties to be compared would be grown.

The F_2 generations in our present cross were invariably grown in very small plots of average fertility. The environmental conditions under which the different plants were grown were more or less uniform and therefore it is believed that relative values were secured of the different measurements of internal tissues to be reported hereunder.

As mentioned previously freehand sections were cut for the study of the internal anatomical characters. Entire or partial sections were cut as thinly as possible and stained with safranin. It was found that the band of sclerenchymatous tissue in type 1 (lodging) was very poorly developed while that in barley type 21 (non-lodging) was very well developed. The intermediate phenotypes, on the other hand, showed fair development of this tissue. For the purpose of these studies the radial width of the sclerenchymatous layers were measured and so was the thickness of the cell-wall taken. All measurements were made under the microscope (Leitz) with eye-piece No. 1 and objective No. 7 and magnification 335×. The measurements given in the following pages were taken with a micrometer eye-piece and the units referred to in Table I, for instance, represent unit divisions of this eye-piece. These figures in Table IV could be converted in terms of microns by multiplying by the constant 2·857 but this appears to be unnecessary for our work.

Inheritance of radial width of sclerenchymatous band

Table IV shows the distribution of F_2 plants and some representative F_3 families for their respective widths of the sclerenchymatous band together with the parental frequencies. The range extends from 7 to 16 units, each unit being equal to $2 \cdot 857\mu$.

It may be pointed out that the segregation of only a few representative F_3 families has been shown in Tables IV-VII in order to save printing space, although a large number of families in each has been studied.

TABLE IV

Frequency distribution of the radial width of the sclerenchymatous band

Culture	Radial width of the sclerenchymatous layer in units											Total No. of plants	Mean	S. Dev.	Coefficient of variation
	F_2 parent	7	8	9	10	11	12	13	14	15	16				
Type 21 parent	..								7	27	11	45	15·09±0·09	0·63±0·07	4·15±0·44
Type 1 parent	..			20	21	4						45	9·64±0·10	0·65±0·07	6·78±0·72
T.21×T.1—F_2	..	1	9	49	101	50	24	10	3	4	2	253	10·40±0·09	1·42±0·06	13·63±0·61
T. 21×T. 1—F_3															
239 . .	12·33								6	13	7	26	15·04±0·09	0·73±0·07	4·85±0·67
230 . .	10·83						3	12	4	7	3	29	13·83±0·22	1·25±0·16	9·04±1·20
231 . .	11·66					3	13	12	10	1		39	12·82±0·11	0·98±0·07	7·64±0·85
65 . .	12·67					5	5	3	2	3	2	20	12·95±0·36	1·69±0·25	13·05±2·10
264 . .	11·33				5	5	7	6				23	11·61±0·15	1·09±0·11	9·39±1·39
220 . .	9·33			5	12	7						24	10·08±0·09	0·70±0·07	6·94±1·01
15 . .	9·80		1	10	12	4						27	9·75±0·12	0·95±0·09	5·64±0·77
262 . .	9·16			8	12	3	2	3	2	1	1	32	10·84±0·35	2·07±0·25	19·10±2·47
70 . .	9·83			4	10	6	2	6	2	2	1	33	11·45±0·31	1·83±0·22	15·98±2·02
5 . . .	10·00			2	13	5	3	4	0	1	1	29	11·10±0·24	1·50±0·18	13·50±1·80

It will be observed that the average radial width of this sclerenchymatous band is 9·644 units in type 1 and 15·089 units in type 21 ; the average width of the 253 F_2 plants studied is 10·403 units and that its range extends below the range of the lower parent. The coefficient of variability also is much higher than that of either parent. The F_2 population is distributed in a somewhat skew manner with the mode towards the parent (type 1) with lesser width (Fig. 2).

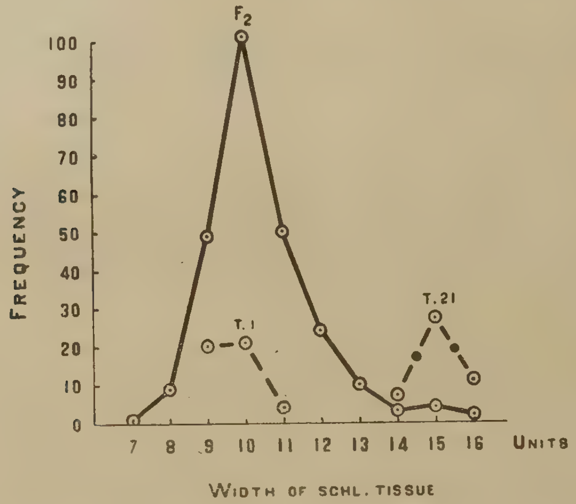


FIG. 2. Frequency distribution of type 21, type 1 and F_2 for the radial width of the Sclerenchymatous band.

In the case of the sclerenchymatous cylinder, therefore, multiple factors appear to be responsible for the inheritance of this character. This is substantiated by the F_3 distributions.

Inheritance of the size of the vascular bundles

That the size of the vascular bundle is also a differentiating character in the internal anatomy of the barley plant may be gauged from the fact that both the long (tangential) and the short (radial) diameters differ significantly in the two parents involved in this study. That the size and the number of vascular bundles in unit area play an important role in relation to lodging has been proved by many workers but the inheritance of the size of the vascular bundles has perhaps not been worked out previously. Measurements were made under a Leitz microscope with a No. 3 objective and eye-piece No. 1, magnification 60× and hence the unit divisions mentioned in the measurements of the tangential and radial axes of the vascular bundles can be converted into microns by multiplying the units by the constant 16·67.

Inheritance of the size of the tangential diameter of the vascular bundles

The following type of segregation for this character was observed :—

TABLE V

Frequency distribution of the tangential diameter of the vascular bundles

Culture	F_2 parent	Size of tangential diameter in units											Total number of plants	Mean	S. Dev.	Coefficient of variation
		7·0	7·5	8·0	8·5	9·0	9·5	10·0	10·5	11·0	11·5	12·0				
Type 21 parent .	..							2	16	22	4	1	45	10·84±0·05	0·39±0·04	3·10±0·33
Type 1 parent .	..			8	17	10	10						45	8·74±0·08	0·51±0·05	5·87±0·62
T. 21×T. 1—F_2.	..	1	3	5	15	42	48	69	38	27	7	1	256	9·80±0·04	0·84±0·04	8·60±0·38
T. 21×T. 1—F_3—																
40 . .	9·67			2	5	19	3	1					30	8·94±0·07	0·40±0·05	4·47±0·58
220 . .	10·00		1	1	2	9	10	1					24	9·10±0·10	0·78±0·07	8·53±1·24
15 . .	..				2	11	3	6	4				26	9·48±0·12	0·63±0·09	6·62±0·92
10 . .	9·33							3	15	10	5		33	10·76±0·08	0·47±0·06	4·35±0·54
271 . .	9·00						4	8	7	4			23	10·24±0·14	0·97±0·09	9·47±1·41
140 . .	9·83						1	7	10	9			27	10·50±0·08	0·43±0·05	4·11±0·56
157 . .	10·00			2	3	9	2	2	2	3			23	9·37±0·19	0·90±0·13	9·62±1·43
262 . .	9·00			5	2	11	4	3	2	4	1		32	9·39±0·18	1·03±0·12	11·02±1·39
218 . .	9·33			4	2	5	6	8	4	3	2		34	9·68±0·16	0·97±0·12	10·01±1·22
185 . .	9·50		2	3	5	8	2	4	5	3			32	9·31±0·21	1·21±0·15	13·03±1·64

The average size of vascular bundles in type 1 is smaller than that of type 21, being 8·744 units in the former and 10·844 units in the latter case. The F_2 covers the whole range of both the parents and in fact exceeds the lower limit of type 1 and the average size is more or less intermediate between the size of the two parents, being 9·802 units. The frequency array indicates an approach to a normal curve and shows that multiple factors are responsible for the inheritance of this character. The F_3 families studied confirm this theory.

Inheritance of the size of the radial diameter of the vascular bundles

In Table VI are presented the frequency distributions of the two parents and their F_2 and some F_3 progenies for the radial diameter of the vascular bundles.

The average size of the radial diameter of the vascular bundles in types 1 and 21 respectively are 7·644 and 9·855 units and that of the F_2 is 9·548 units. The range of type 1 is rather large but the F_2 covers the complete range of both the parents and the form of the frequency array definitely shows that multiple factors are responsible for the inheritance of this character. This is substantiated by the distributions of the F_3 families.

STUDIES ON CORRELATED INHERITANCE

In the foregoing pages we have considered the inheritance of a single variable taken separately but it is also important to see whether any of these is interrelated with any other variable and for this purpose we use the method of correlation. If one character is associated with any other character it must show correlation or linkage and the detection of linkage relations between morphological and anatomical characters is of very great economic importance.

TABLE VI

Frequency distribution of the radial diameter of the vascular bundles

Culture	F_2 parent	Size of radial diameters in units										Total number of plants	Mean	S. Dev.	Coefficient of variation
		6·0	6·5	7·0	7·5	8·0	8·5	9·0	9·5	10·0	10·5				
Type 21 parent .	..							2	10	32	1	45	9·86±0·04	0·29±0·03	2·94±0·31
Type 1 parent .	..	2	3	9	11	8	10	2				45	7·64±0·11	0·75±0·08	9·82±0·79
T. 21×T. 1—F_2 .	..	1	3	16	34	46	71	44	30	10	1	256	8·42±0·05	0·80±0·04	9·50±0·42
T. 21×T. 1—F_3—															
220 . .	8·16		1	1	5	11	6					24	7·92±0·10	0·49±0·07	6·19±0·89
15 . .	8·00			2	4	16	4					26	7·92±0·09	0·46±0·06	5·81±0·22
40 . .	8·17			4	4	11	10	1				30	8·00±0·10	0·53±0·07	6·63±0·86
270 . .	9·16						3	19	6	9		37	9·28±0·08	0·48±0·06	5·17±0·60
239 . .	10·33							4	9	13	1	27	9·70±0·06	0·43±0·05	4·43±0·60
18 . .	10·25							1	9	11	4	25	9·86±0·08	0·39±0·05	3·95±0·56
70 . .	7·50			4	2	10	5	8	1	3		33	8·39±0·14	0·83±0·10	9·89±1·23
176 . .	7·83			2	4	7	9	5	2	2		31	8·40±0·14	0·76±0·10	9·04±1·16
200 . .	8·33			2	3	6	6	14	1	3		35	8·60±0·13	0·75±0·09	8·72±1·05
218 . .	8·00		1	3	4	7	6	9	2	2		34	8·37±0·15	0·89±0·11	10·64±1·20

Correlation between the radial width of the sclerenchymatous band and the thickness of the sclerenchymatous cell-wall in culms of barley cross type 21×type 1—F_2

This relationship is represented in the following correlation surface :—

TABLE VII

Thickness of the sclerenchymatous cell-wall—*Y*—	Radial width of sclerenchymatous band—*X*—										Total—*Y*—
	7	8	9	10	11	12	13	14	15	16	
0·6	1	3	1								5
0·7		3	11	4							18
0·8		3	20	24	11						58
0·9			16	57	19	11	1				104
1·0			1	16	19	9	7	2	1		55
1·1						3	2	1	2		8
1·2					1	1				2	4
1·3											0
1·4									1		1
Total—*X*— .	1	9	49	101	50	24	10	3	4	2	253

The coefficients of correlation and their transformed z values for the F_2 as well as for the two parents are as follows :—

	r_{xy}	z	Std. error of z	Ratio of $\frac{z}{S.E._z}$
Type 21 × type 1—F_2 . . .	0·6910	0·85	0·0629	13·51
Type 21 parent	0·4935	0·54	0·1543	3·50
Type 1 parent	0·7901	1·08	0·1543	6·99

It is evident from the value of the correlation coefficient as well as from the scatter of the variables in the correlation table that a high positive correlation exists between the radial width of the sclerenchymatous band and the thickness of the sclerenchymatous cell-wall. In other words, both factors for the strength of the culm are correlated and an increase in the width of the sclerenchymatous band corresponds to an increase in the thickness of the cell-wall.

Correlation between the radial and tangential diameters of the vascular bundles in the culms of barley type 21 × type 1—F_2

The association of these two characters is represented in the following table :—

TABLE VIII

Tangential diameter—y—	Radial diameter—x—										Total—y—
	6·0	6·5	7·0	7·5	8·0	8·5	9·0	9·5	10·0	10·5	
7·0 . . .	1										1
7·5 . . .		2	1								3
8·0 . . .			5								5
8·5 . . .		1	3	9	2						15
9·0 . . .			5	16	16	5					42
9·5 . . .			1	6	17	22	2				48
10·0 . . .			1	2	6	31	21	8			69
10·5 . . .				1	5	9	10	8	5		38
11·0 . . .						4	10	12	1		27
11·5 . . .							1	2	3	1	7
12·0 . . .									1		1
Total—x— .	1	3	16	34	46	71	44	30	10	1	256

The coefficients of correlation and their respective z values are given hereunder :—

	r_{xy}	z	Std. error of z	Ratio of $\frac{z}{\text{S. E.}_z}$
Type 21 × type 1—F_2 . . .	0·8043	1·11	0·0625	17·76
Type 21 parent	0·4140	0·44	0·1543	2·85
Type 1 parent	0·5290	0·59	0·1543	3·18

A high positive correlation exists between the tangential and radial diameters of the vascular bundles.

In Tables IX and X are shown the correlation of quantitative characters with each other. The high degree of correlation observed in each comparison indicates that only one set of factors is operating in every case.

Correlation between the radial width of the sclerenchymatous band and the tangential diameter of the vascular bundles in the culms of barley type 21 × type 1—F_2

That these two characters are correlated can be recognised from the distribution of the arrays in the following table :—

TABLE IX

Tangential diameter—y—	Radial width of the sclerenchymatous band—x—										Total—y—
	7	8	9	10	11	12	13	14	15	16	
7·0 . . .	1										1
7·5 . . .		3									3
8·0 . . .		1	2	2							5
8·5 . . .		1	6	6	2						15
9·0 . . .		2	25	10	3	2					42
9·5 . . .		2	6	25	12	2	1				48
10·0 . . .			9	34	20	4	2				69
10·5 . . .			1	18	7	7	3	1	1		38
11·0 . . .				5	9	8	3		1	1	27
11·5 . . .				1		1	1	2	2		7
12·0 . . .										1	1
Total—x— .	1	9	49	101	53	24	10	3	4	2	256

Coefficients of correlation and their transformed z values were as follows :—

	r_{xy}	z	Std. error of z	Ratio of $\frac{z}{\text{S. E.}_z}$
Type 21 × type 1—F_2	0·5331	0·59	0·0625	9·44
Type 21 parent	0·4970	0·55	0·1543	3·56
Type 1 parent	0·6476	0·77	0·1543	4·99

Positive correlation between the two characters is again indicated.

Correlation between the radial width of the sclerenchymatous band and the radial diameter of the vascular bundles in the culms of barley type 21×type 1—F_2

A positive correlation is also apparent between these two characters and is brought out below :—

TABLE X

Radial diameter	Radial width of the sclerenchymatous band –*x*–										Total –*y*–
	7	8	9	10	11	12	13	14	15	16	
6·0 . . .		1									1
6·5 . . .	1	2									3
7·0 . . .		3	6	7							16
7·5 . . .		1	16	17							34
8·0 . . .		1	18	20	2	3	2				46
8·5 . . .		1	9	36	21	2	1		1		71
9·0 . . .				16	20	5	1	1		1	44
9·5 . . .				5	10	12	3				30
10·0 . . .						2	2	2	3	1	10
10·5 . . .							1				1
Total –*x*– .	1	9	49	101	53	24	10	3	4	2	256

Coefficients of correlation and their respective *z* transformations were as shown hereunder :—

	r_{xy}	z	Std. error of z	Ratio of $\frac{z}{\text{S. E.}_z}$
Type 21×type 1—F_2 . .	0·726	0·92	0·0625	14·72
Type 21 parent . . .	0·557	0·63	0·1543	4·08
Type 1 parent . . .	0·433	0·46	0·1543	2·98

In the tables which are to follow we will consider the relation of the nature of the mechanical tissue to quantitative characters such as the width of the sclerenchyma in the F_2 plants of the cross under discussion.

Correlation between the nature of the mechanical tissue with the radial width of the sclerenchymatous band

This association may be gauged from the following distribution :—

TABLE XI

Phenotypes –y–	Genetic constitution	Radial width of the sclerenchyma –x–										Total –y–
		7·0	8·0	9·0	10·0	11·0	12·0	13·0	14·0	15·0	16·0	
Like type 1	AB	1	8	48	42	31	12					142
Inter.-A .	Ab			3	21	14	7	3				48
Inter.-B .	aB			5	15	20	7	2				49
Like type 21.	ab					2	0	3	4	3	2	14
Total –x–	..	1	8	56	78	67	26	8	4	3	2	253

The range of F_2 plants, resembling the type 1 phenotype, extends from seven to twelve units, while that of plants like type 21, goes from eleven to sixteen units. The range of Inters. A and B lies between nine and thirteen or almost in an intermediate position between the ranges of the two parental phenotypes, and they are more or less similar to each other. This shows that there is a definite association of the phenotypes for the degree and distribution of mechanical tissue and the radial width of the sclerenchymatous layers.

Correlation between the degree and distribution of the mechanical tissues with the thickness of the sclerenchymatous cell-wall

The relationship between these two characters may be seen from the table given below :—

TABLE XII

Phenotypes –y–	Genetic constitution	Thickness of the cell-wall –x–									Total –y–
		0·6	0·7	0·8	0·9	1·0	1·1	1·2	1·3	1·4	
Like type 1 .	AB	5	8	36	68	23					140
Inter.-A . .	Ab	1	0	6	28	12	2				49
Inter.-B . .	aB	1	3	8	17	12	5				46
Like type 21 .	ab					2	7	4	0	1	14
Total –x– .	..	7	11	50	113	49	14	4	0	1	249

In this case it will be observed that the range of phenotypes susceptible to complete or partial lodging, *i.e.*, of type 1 and Inters. A and B, range from 0·6 to 1·1 units, while that of the phenotype which is resistant to lodging (*i.e.*, like type 21), extends from 1·0 to 1·4. It is evident that the F_2 plants which resembled the type 21 parent in the nature of its mechanical tissue had a thicker cell-wall also.

Correlation between the degree and distribution of the mechanical tissue with the diameter of the tangential axis of the vascular bundles

The following table depicts the relationship of these two characters :—

TABLE XIII

Phenotypes –y–	Genetic constitution	Size of the tangential diameter –x–											Total –y–
		7·0	7·5	8·0	8·5	9·0	9·5	10·0	10·5	11·0	11·5	12·0	
Like type 1.	AB	1	3	6	11	32	34	44	12				143
Inter.-A .	Ab				2	4	9	13	13	7			48
Inter.-B .	aB				3	5	5	12	12	11	1		49
Like type 21.	ab									6	4	4	14
Total –x–	..	1	3	6	16	41	48	69	37	24	5	4	254

The data presented in the above table also brings out the fact that the diameter of the tangential axis of the vascular bundles has a range of from seven to twelve units in phenotype like type 1, and of 8·5 to 11·5 units in the two intermediate phenotypes, whereas the phenotype resistant to lodging, *viz.*, that like type 21, has a range of from eleven to twelve units. In other words, the greater the size of the tangential diameter of the vascular bundles the more resistant is the plant to lodging.

Correlation between the nature of the mechanical tissue with the diameter of the radial axis of the vascular bundles

The relationship between these two characters is presented in the following table :—

TABLE XIV

Phenotypes –y–	Genetic constitution	Size of the radial diameter –x–										Total –y–
		6·0	6·5	7·0	7·5	8·0	8·5	9·0	9·5	10·0	10·5	
Like type 1.	AB	1	2	15	20	44	32	24	3			141
Inter -A .	Ab			2	3	9	12	11	9	2		48
Inter -B .	aB			2	2	7	12	17	8	1		49
Like type 21.	ab							2	3	7	2	14
Total –x–	..	1	2	19	25	60	56	54	23	10	2	252

As in the other cases, here too a definite relationship between the degree and distribution of mechanical tissue found in the different phenotypes and the size of the radial diameter of the vascular bundles is apparent. The range of plants like type 1 extends from 6·0 to 9·5 units, that of Inters. A and B from 7·0 to 10·0 units while that of plants like type 21 goes from 9·0 to 10·5 units.

Correlation between the nature of the mechanical tissue with the fertility of plants in F_2

There appears to be no correlation between the anatomical phenotypes and those for the fertility of the ear-head. This will be apparent from the observed and expected figures shown under each phenotype, the latter figures being calculated on the basis of independent segregation. The ratios, however, are somewhat distorted owing to certain casualties in this population due to the attack of termites.

TABLE XV

Fertility of the ear-head	Frequencies	Anatomical phenotypes				Total
		Like T. 1	Inter.-A	Inter.-B	Like T. 21	
2-rowed .	Observed .	27	13	18	2	60
	Expected .	33·75	11·25	11·25	3·75	
Intermediate	Observed	73	22	22	6	123
	Expected .	69·19	23·06	23·06	7·69	
6-rowed .	Observed .	48	18	9	7	82
	Expected .	46·13	15·37	15·37	5·13	
	χ^2	2·32	0·77	6·74	1·85	11·68
	$P >$	0·20	0·50	0·02	0·30	0·30

Similarly no correlation was found to exist between the anatomical phenotypes and (1) width of outer glume, (2) length of ear-head, (3) tillering capacity of plants and (4) maturity of the plants.

Lodging depends on the interaction of so many complex factors, both environmental and genetical, that a naked-eye estimate of the behaviour of plants for this character can furnish only a rough, relative measure of the phenomenon. The genetic nature of the plant for this character can therefore be better understood by the examination of the degree and development of the internal mechanical tissue. This, however, is not a very easy task for the plant-breeder to accomplish with the thousands of plants which he has to deal with. He has, therefore, to satisfy himself with external examination of plant structures alone and to base his selections on these studies. After making a preliminary selection of a few plants for resistance to lodging he can easily confirm his opinion by detailed internal examination of the main anatomical features of his selected individuals.

The stand of 263 F_3 families raised from the present cross, type 21 × type 1, was observed sometime before harvesting, and careful notes were taken regarding the extent of lodging in each family. A classification of the plants according to four grades of lodging and one of non-lodging was made and these observations were later correlated with the actual anatomical phenotypic behaviour of each family. The relationship observed is tabulated below :—

TABLE XVI

Relation of lodging behaviour with anatomical phenotypes

Lodging behaviour	Anatomical phenotypes				Total
	Like T. 1	Inter.-A	Inter.-B	Like T. 21	
L = fully lodged . .	64	5	3	0	72
L_1 = three-fourths lodged .	59	24	3	0	86
L_2 = half lodged . .	24	17	13	0	54
L_3 = stray plants lodged .	0	5	28	5	38
l = non-lodged . . .	0	0	3	10	13
Total .	147	51	50	15	263

The results are obvious and do not want any comment beyond the fact that plants with internal anatomy like type 1 and those like Inters. A and B show different grades of lodging while those in which the mechanical tissue is like type 21 are either entirely non-lodging or show but a few stray lodged plants.

SUMMARY AND CONCLUSIONS

A cross between two Pusa barleys, type 21 × type 1, was studied genetically for the inheritance of some anatomical characters in relation to lodging. Type 21 possesses strong straw and type 1 has rather weak straw. No previous work of this nature appears to have been published on the mode of inheritance of internal anatomical characters responsible for the lodging of cereals.

The problem of lodging is too complex to depend on any one single factor, but that anatomical structures do play a very important rôle in lodging is a fact that cannot be contested. The present studies show that the development and inheritance of these mechanical tissues, like any other external character, are controlled by Mendelian factors.

Material was collected from parental lines and from F_2 and F_3 populations after internodal growth had completely ceased, but before the grain was actually ripe. A piece about 3 cm. in length was cut at the third internode from the top of the main culm of each plant and preserved for a couple of months in formalin alcohol. Freehand cross-sections were taken of each and stained with safranin.

Four anatomical phenotypes were recognised according to the arrangement and position of the peripheral sclerenchyma :—

Phenotype	Arrangement and position of sclerenchyma
Like type 1 . . .	Very close to the periphery and poorly developed.
Inter.-A . . .	Generally close to the periphery and well developed.
Inter.-B . . .	Mostly away from the periphery and connected at places by irregularly-placed sub-epidermal girders ; mechanical tissue well developed.
Like type 21 . .	Away from the periphery and connected to it at regular intervals by well-developed sub-epidermal girders ; mechanical tissue very well developed.

At least two genetic factors appear to be responsible for the inheritance of the degree of development and distribution of the sclerenchyma. Factor **A** represents a dominant factor for susceptibility to lodging and **B** a supplementary factor which is less potent than **A** in effect, but which in the presence of this factor induces complete lodging. The recessive allelomorphs, **a** and **b** condition resistance to lodging. Under these circumstances barley type 1 may be represented to possess the genetic constitution **AABB**, while type 21 may have **aabb**. Inter.-A will then have the constitution, **AAbb** and Inter.-B will have **aaBB**. The F_2 segregation is of the type 9 **AB** : 3 **Ab** : 3 **aB** : 1 **ab**.

The following quantitative characters studied individually showed that multiple factors were responsible for their inheritance :—

1. Radial width of the sclerenchymatous band.
2. Diameter of the tangential axis of the vascular bundles, and
3. Diameter of the radial axis of the vascular bundles.

All the three characters detailed above showed definite association with the degree and distribution of the mechanical tissue as represented by the four anatomical phenotypes and it is possible that only one set of genetical factors is operating in all the characters considered.

The undermentioned characters showed definite positive correlation between themselves :—

1. Width and thickness of sclerenchymatous tissue.
2. Tangential and radial diameters of the vascular bundles.
3. Radial width of the sclerenchyma and the tangential diameter of the vascular bundles.
4. Radial width of the sclerenchyma and the radial diameter of the vascular bundles.

No correlation was found to exist between the anatomical phenotypes and (1) fertility of the ear-head, (2) width of outer glumes, (3) length of ear-head, (4) tillering capacity of plants, and (5) maturity of the plants.

Part II.— The inheritance of some ear-head characters

Because of its economic importance, its many easily-determinable heritable characters and the small number of chromosomes, the barley plant affords good material for genetical studies.

The fertility of the lateral florets in the barley ear is taken as a criterion of separating the fully fertile, six-rowed species from the two-rowed one in which the lateral florets are always abortive or sterile. Most investigators have found that this sterility is dominant to the other allelomorph and observed that the difference between the two depends on the interaction of a single factor, giving a simple 3 : 1 ratio [Biffen, 1907 ; Gaines, 1917 ; Wexelsen, 1934 ; etc.]. The intermediate type of fertility as represented by the F_1 hybrids has mostly been considered to be of hybrid origin, but Harlan and Hayes [1920] were able to obtain for the first time the homozygous form of *H. intermedium* from a cross between Manchuria, a six-rowed barley and Svanhals, a two-rowed variety.

The inheritance of the character for awn development has been found by many workers to depend on a single factor only. Broad glumes were found to be a simple recessive character by Biffen [1907], but Schiemen [1921] and Hor [1924] have recorded the appearance of the broad-glumed type in the F_3 generation of crosses of the normal narrow-glumed forms. A very handy list of references on the subject is available in the barley breeding bibliography of the Imperial Bureau of Plant Genetics, Cambridge [1931].

F_1 GENERATION

Only two plants were raised in this generation and both of them showed great hybrid vigour as evidenced by the very high number of tillers, yield of seed and by their vigorous growth. The ear-heads were longer than those in either parent and had a tendency to droop down slightly on maturity. The fertility was of an intermediate type and in a few cases some lateral florets were observed to be fertile. The intermediate type of ear-head could be differentiated from the two-rowed ear-head in having pointed and not blunt palea in the lateral florets as will be seen in Plate II, fig. 2, in contrast to fig. 4 of the same plate.

The differentiating characters used in the present studies are tabulated below :—

TABLE XVII

Differentiating characters

Characters	Type 1 parent	F_1 hybrids	Type 21 parent
1. Fertility of the lateral florets	Sterile giving 2-rowed ears.	Intermediate and partially fertile ears.	Fully fertile giving 6-rowed ears.
2. Awns on the inner glumes of the lateral florets .	*Nil*	Intermediate, pointed or tipped	Fully developed
3. Nature of outer glumes .	Broad . .	Narrow .	Narrow.

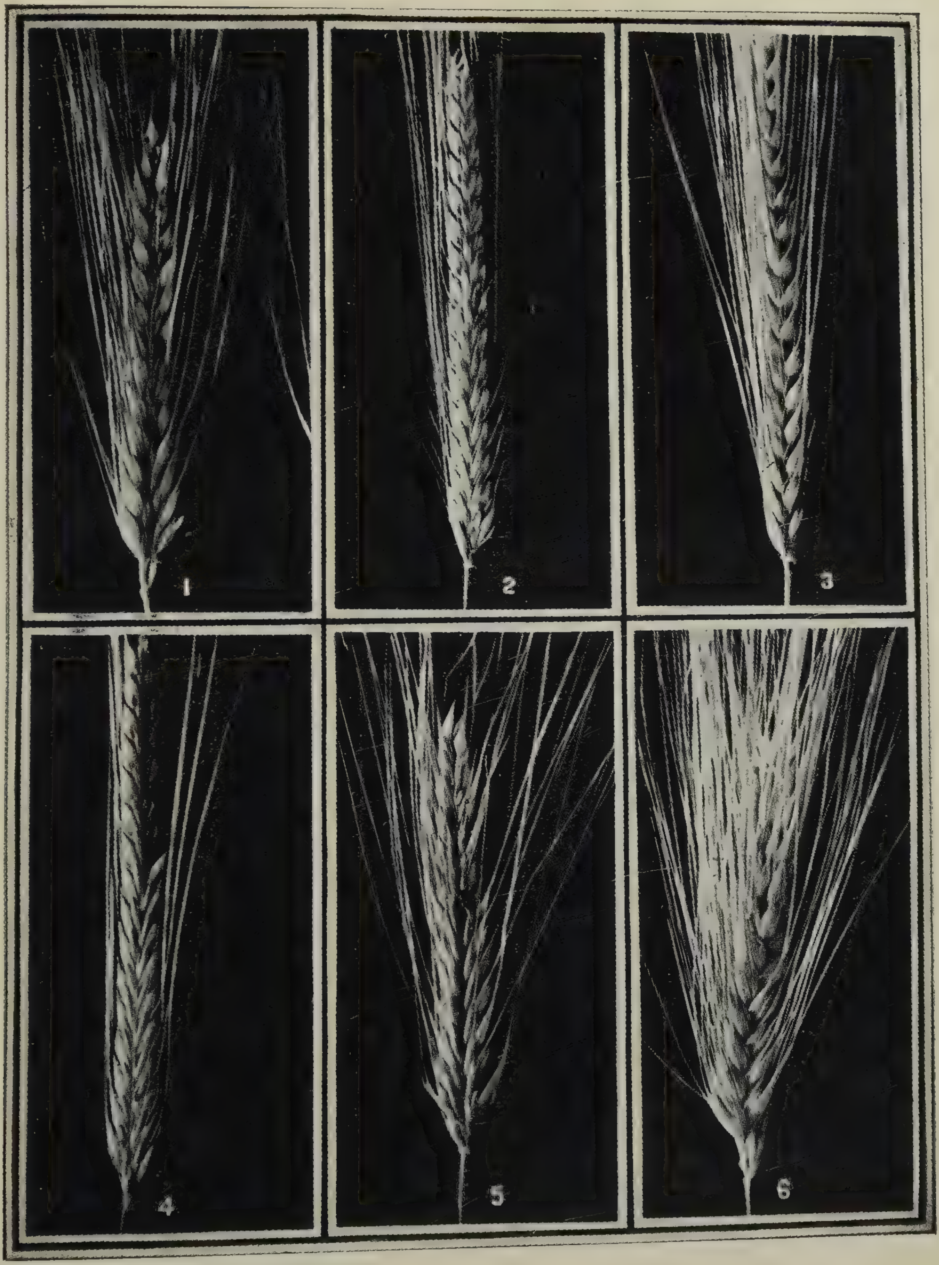
It will be noted that the F_1 hybrids were intermediate in character for fertility and awn development and showed complete dominance of narrow outer glumes.

F_2 AND F_3 GENERATIONS

The F_2 and F_3 generations were grown under the usual field conditions in fields of average fertility. In 1932-33 two series of F_2 were grown. In series B, untreated seed was dibbled by hand whereas in series A the seed was disinfected with universal uspulun, 0·25 per cent before dibbling, in order to minimize the effects of *Helminthosporium* which is a very common disease of barley at Pusa. In 1933-34 a further lot of F_2s was grown from the same F_1 seed and is referred to below as series C.

INHERITANCE OF FERTILITY OF THE LATERAL FLORETS AND OF AWN DEVELOPMENT

As has been mentioned above type 1 is a two-rowed barley, while type 21 is a six-rowed form. The awns on the outer palea of the lateral florets are fully developed in type 21 and altogether absent in type 1. The fertility of the lateral florets and the development of awns on their outer palea have been observed to be perfectly linked and their inheritance has been found to depend on the interaction of the same genetic factors. The F_1 showed an intermediate type of ear-head and



Type of ear-heads observed in F_2 in a cross between Pusa barleys, type 21 × type 1.

1. 2-rowed barley, like type 1 parent, with broad outer glumes and blunt palea in the lateral florets.
2. Intermediate barley like F_1 with narrow outer glumes and pointed lateral florets.
3. 6-rowed barley, like type 21 parent, with narrow outer glumes and pointed lateral florets.
4. 2-rowed barley with narrow outer glumes and blunt palea in the lateral florets.
5. Intermediate barley with broad outer glumes and pointed palea in the lateral florets.
6. 6-rowed barley with broad outer glumes and pointed palea in the lateral florets.

It may be pointed out that in Fig. 6 the outer glumes of each of the three florets in each row are broad and fully awned resulting in the development of nine awns in each row in contrast to three awns found in ordinary 6-rowed barleys.

tipped lateral florets and the F_2 showed the following phenotypes and segregations :—

TABLE XVIII

Segregation in F_2 for fertility of the lateral florets and of awn development on the inner glumes of the lateral florets on a 1 : 2 : 1 basis

Series	Year	Observed frequencies			Total No. of plants	χ^2	P between
		Fertility → 2-rowed	Inter.	6-rowed			
		Awns on lateral florets → *Nil*	Inter.	Full			
A	1932-33	60	134	83	277	3·91	0·1—0·2
B	1932-33	60	145	61	266	2·17	0·3—0·5
C	1933-34	59	109	58	226	0·29	0·8—0·9
	Total	179	388	202	769	1·43	0·3—0·5

Owing to an excess of six-rowed plants the fit between observed and expected frequencies is not very good in series A, but in the other two series and in the final total the fit is quite close and may be considered as very satisfactory. The fit in series C specially is very good indeed. It may be pointed out that there were a large number of casualties in series A due to white ants and this may be responsible for the distorted ratios observed.

These figures clearly suggest that there is a single factor difference between the two-rowed and six-rowed phenotypes in this case. This is further substantiated by the F_3 behaviour brought out in the following table. It may be pointed out that the progeny of all F_2 plants in series A was grown in the following year in order to study the F_3.

TABLE XIX

F_3 behaviour for the inheritance of the fertility of the lateral florets

No. of families	Nature of F_2 phenotype	Genotypic constitution	F_3 behaviour	No. of families	
				Observed	Expected
60	2-rowed	ZZ	Pure 2-rowed	60	60
134	Intermediate	Zz	Pure 2-rowed	1	0 Wrongly diagnosed in F_2
			Segregating(1 : : 1)	133	134
83	6-rowed	zz	Pure 6-rowed	83	83

The 133 segregating F_3 families showed the frequencies detailed in Table XX. The F_3 study fully confirms the F_2 hypothesis that fertility of lateral florets depends on a single factor difference.

TABLE XX

Behaviour of segregating F_3 families for the inheritance of the fertility of and the awn development on the lateral florets

Culture No.	Observed frequency			Total No. of plants	χ^2	*P* between	Culture No.	Observed frequency			Total No. of plants	χ^2	*P* between
	A	B	C					A	B	C			
1	13	30	10	53	1·209	0·50-0·70	69	12	22	7	41	1·439	0·30-0·50
2	14	32	13	59	0·458	0·70-0·80	72	16	26	14	56	0·429	0·80-0·90
3	13	31	14	58	0·310	0·80-0·90	74	13	26	18	57	1·791	0·30-0·50
4	14	26	15	55	0·200	0·90-0·95	76	9	35	15	59	3·271	0·10-0·20
5	7	26	7	40	3·600	0·10-0·20	77	15	26	18	59	1·119	0·50-0·70
6	13	33	18	64	0·844	0·50-0·70	78	17	26	23	66	3·956	0·10-0·20
9	19	25	11	55	2·780	0·20-0·30	80	13	36	13	62	1·640	0·30-0·50
10	7	20	11	38	1·431	0·30-0·50	85	13	37	20	70	1·628	0·30-0·50
12	8	20	9	37	0·385	0·80-0·90	87	13	20	5	38	3·474	0·10-0·20
22	11	26	10	47	0·574	0·70-0·80	90	11	24	16	51	3·296	0·10-0·20
23	19	26	14	59	2·013	0·30-0·50	91	17	27	10	54	1·816	0·50-0·70
24	7	34	10	51	6·019	0·02-0·05	93	16	34	18	68	0·118	0·90-0·95
31	20	30	17	67	0·934	0·50-0·70	96	12	29	12	53	0·472	0·70-0·80
36	13	26	16	55	0·491	0·70-0·80	97	8	19	16	43	3·558	0·10-0·20
37	10	33	15	58	1·965	0·30-0·50	98	12	29	14	55	0·309	0·20-0·30
39	18	21	13	52	2·885	0·20-0·30	101	13	25	15	53	0·321	0·20-0·30
41	15	33	23	71	2·155	0·30-0·50	102	14	41	15	70	2·086	0·30-0·50
46	13	40	19	72	1·889	0·30-0·50	104	11	15	13	39	2·282	0·30-0·50
48	11	33	17	61	1·580	0·30-0·50	105	13	30	17	60	0·533	0·70-0·80
50	7	20	16	43	3·977	0·10-0·20	109	15	27	17	59	0·559	0·70-0·80
51	0	5	2	7	0·678	0·70-0·80	111	15	30	20	65	1·154	0·50-0·70
52	9	23	14	46	1·087	0·50-0·70	116	13	40	15	68	2·235	0·30-0·50
53	13	30	18	61	0·836	0·50-0·70	117	13	22	14	49	0·551	0·70-0·80
54	15	36	19	70	1·314	0·50-0·70	121	14	33	15	62	0·290	0·80-0·90
55	14	36	12	62	1·742	0·30-0-50	124	10	30	16	56	1·571	0·30-0·50
57	9	28	12	49	1·364	0·50-0·70	125	18	33	18	69	0·132	0·90-0·95
59	14	31	12	57	0·579	0·70-0·80	126	17	26	17	60	1·067	0·50-0·70
60	14	27	10	51	0·804	0·50-0·70	129	16	22	19	57	3·237	0·10-0·20
65	12	32	12	56	1·143	0·50-0·70	130	10	31	11	52	1·962	0·30-0·50
66	13	29	17	59	0·559	0·70-0·80	131	11	24	16	51	1·410	0-30-5·00
67	13	40	18	71	1·845	0·30-0·50	132	9	20	12	41	0·460	0·70-0·80
68	10	30	16	56	1·471	0·30-0·50	133	15	41	18	74	1·108	0·50-0·70

TABLE XX—*concld.*

Behaviour of segregating F_3 families for the inheritance of the fertility of and the awn development on the lateral florets—concld.

Culture No.	Observed frequency			Total No. of plants	χ^2	P between	Culture No.	Observed frequency			Total No. of plants	χ^2	P between
	A	B	C					A	B	C			
137	10	33	18	61	2·508	0·20-0·30	205	11	28	16	55	0·927	0·50-0·70
140	12	36	15	63	1·571	0·30-0·50	206	12	29	13	54	0·333	0·80-0·90
141	13	19	18	50	3·880	0·10-0·20	207	15	29	13	57	0·789	0·50-0·70
142	13	31	10	54	1·518	0·30-0·50	209	13	25	16	54	0·630	0·70-0·80
143	8	22	15	45	2·199	0·30-0·50	212	12	36	20	68	2·118	0·30-0·50
144	19	26	20	65	2·631	0·20-0·30	216	12	23	15	50	0·680	0·70-0·80
145	13	30	13	56	0·177	0·90-0·95	217	17	35	16	68	0·088	0·95-0·98
145-A	10	29	12	51	1·057	0·50-0·70	220	19	33	16	68	0·323	0·80-0·90
146	15	24	12	51	0·529	0·70-0·80	222	7	33	15	55	4·527	0·10-0·20
147	10	25	12	47	0·362	0·80-0·90	225	12	27	15	54	0·233	0·80-0·90
149	1	5	1	7	1·286	0·50-0·70	226	15	28	14	57	0·053	0·95-0·98
151	16	20	14	50	1·550	0·30-0·50	228	12	32	11	55	1·182	0·50-0·70
154	10	19	12	41	0·415	0·80-0·90	233	15	20	9	44	2·000	0·30-0·50
155	14	29	11	54	0·796	0·50-0·70	237	15	28	24	67	4·224	0·10-0·20
156	12	36	15	57	0·472	0·70-0·80	242	7	23	18	48	5·125	0·05-0·10
157	12	34	17	63	1·121	0·50-0·70	243	12	32	19	63	1·571	0·30-0·50
160	13	26	14	53	0·142	0·90-0·95	244	15	31	15	61	0·090	0·95-0·98
162	16	30	12	58	0·969	0·50-0·70	246	12	38	13	63	2·714	0·20-0·30
163	9	30	8	47	3·638	0·10-0·20	247	11	23	18	52	2·577	0·20-0·30
169	6	25	11	42	2·714	0·20-0·30	248	14	27	15	56	0·107	0·90-0·95
170	11	32	10	53	2·321	0·30-0·50	250	14	29	15	58	0·034	0·98-0·99
172	15	30	22	67	2·129	0·30-0·50	251	16	26	13	55	0·490	0·70-0·80
180	15	40	20	75	1·000	0·50-0·70	252	5	12	8	25	0·760	0·50-0·70
184	10	26	17	53	1·868	0·30-0·50	254	7	39	12	58	7·759	0·02-0·05
189	14	21	14	49	0·999	0·50-0·70	258	11	37	16	64	2·344	0·80-0·90
191	10	23	12	45	0·156	0·90-0·95	260	11	25	12	48	0·125	0·90-0·95
192	12	24	12	48	0	1·00	263	13	43	10	66	6·230	0·02-0·05
193	10	30	16	56	1·572	0·30-0·50	264	12	18	13	43	1·134	0·50-0·70
194	16	30	16	62	0·064	0·95-0·98	265	8	22	14	44	1·182	0·50-0·70
195	12	28	10	50	0·880	0·50-0·70	268	14	28	13	55	0·018	0·99-1·00
196	15	39	13	67	1·925	0·30-0·50	271	13	21	10	44	0·909	0·50-0·70
197	18	34	20	72	0·333	0·80-0·90	273	9	16	12	37	1·162	0·50-0·70
199	15	28	16	59	0·186	0·90-0·95	274	9	19	8	36	0·167	0·90-0·95
200	19	31	19	69	0·657	0·70-0·80	295	17	35	14	66	0·515	0·70-0·80
204	10	25	18	53	2·585	0·20-0·30							

The fit between observed and expected ratios is quite good in most cases. This fact is proved by Table XXI which shows the observed and expected frequency distributions of the χ^2 values obtained above and Fig. 3 indicates the same graphically.

TABLE XXI

Observed and expected χ^2 distributions of the F_3 families segregating for fertility and awn development of the lateral florets

^{2}	Observed	Expected
0	2	1·33
0·020	1	1·33
0·040	4	3·99
0·103	10	6·65
0·211	11	13·30
0·466	18	13·30
0·713	30	26·60
1·386	31	26·60
2·408	10	13·30
3·219	12	13·30
4·605	1	6·65
5·991	3	3·99
7·824	0	1·33
9·210	0	1·33
Total	133	133

Total χ^2 = 13·9799, P — between 0·30 and 0·50.

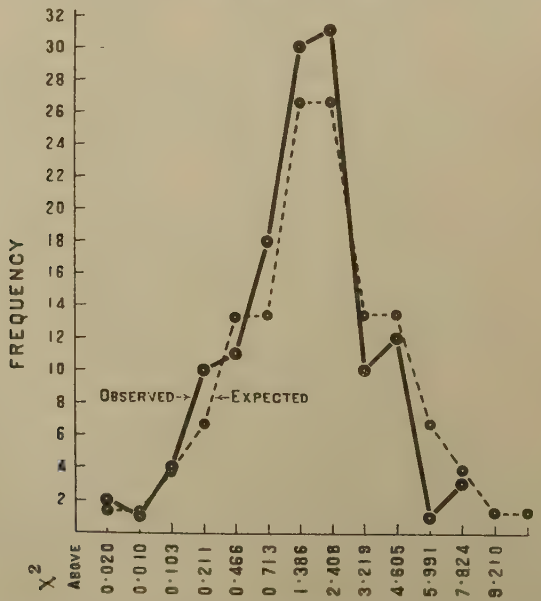
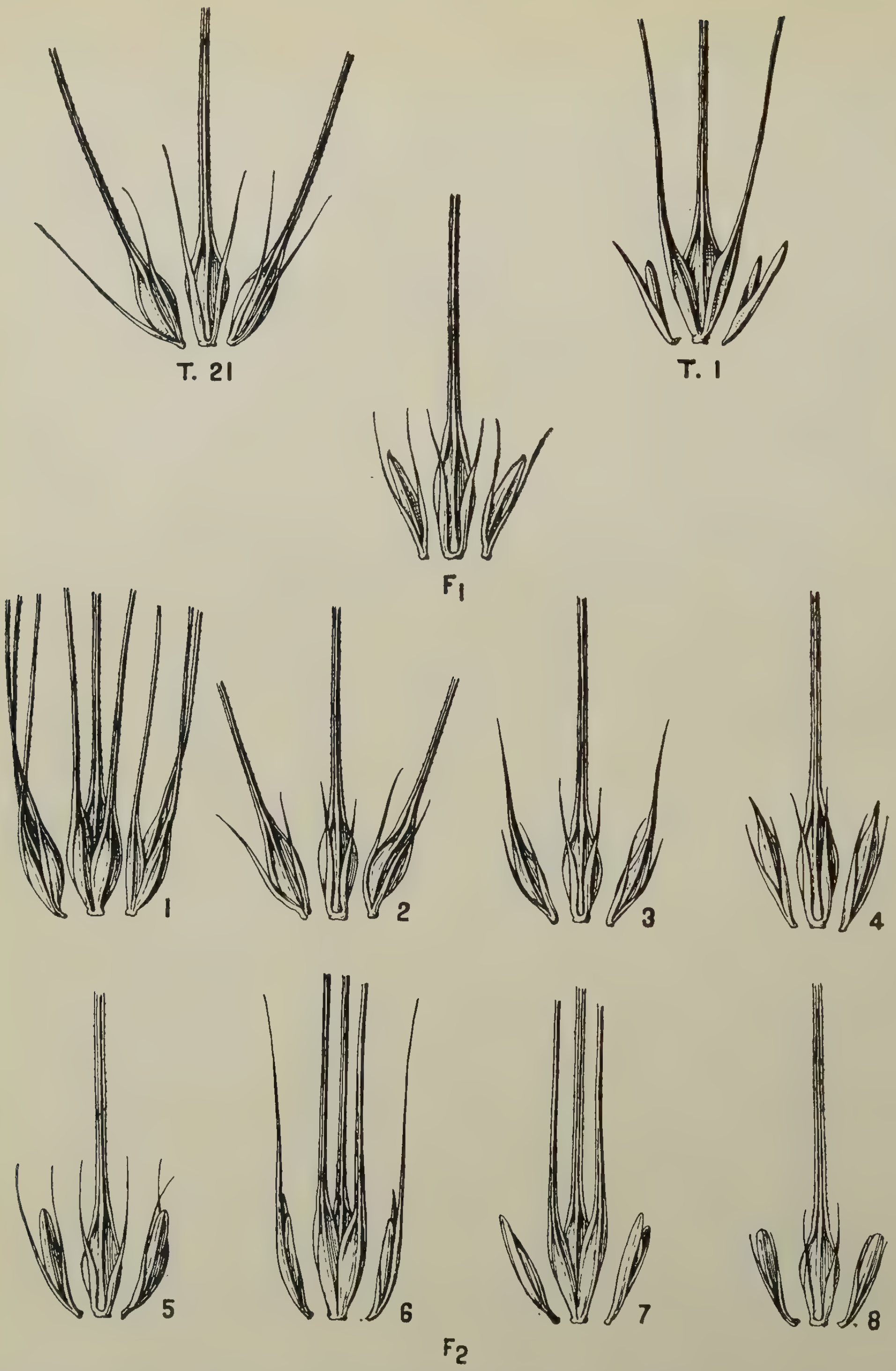


FIG. 3. χ^2 distribution for F_3 families segregating for fertility and nature of awn development on lateral florets in barley cross type 21 × type 1.



Inheritance of fertility of lateral florets and the nature of the outer glumes in a cross between Pusa barleys, type 21 × type 1.

Note the fertile lateral florets and narrow outer glumes in type 21 parent, and the aborted lateral florets and broad outer glumes of type 1 parent. The F_1 is intermediate for fertility while the outer glumes are narrow. The F_2 shows the following grades and are illustrated above :—

1. Six-rowed fertility and broad outer glumes. *2.* Six-rowed fertility and narrow outer glumes. *3.* and *5.* Intermediate fertility and narrow outer glumes. *4.* and *6.* Intermediate fertility and broad outer glumes. *7.* Two-rowed fertility and broad outer glumes. *8.* Two-rowed fertility and narrow outer glumes.

INHERITANCE OF OUTER GLUMES

Type 1 differed from all other Pusa barleys in possessing broad outer glumes while type 21 had narrow outer glumes (Plate III). The F_1 hybrids showed complete dominance of narrow glumes and F_2 segregated on a monogenic basis as is shown below :—

TABLE XXII

F_2 segregation for the nature of outer glumes on a 3 : 1 basis

Series	Year	Observed frequencies		Total No. of plants	χ^2	P between
		Narrow	Broad			
A	1932-33	203	74	277	0·435	0·50—0·70
B	1932-33	205	61	266	0·608	0·30—0·50
C	1933-34	170	56	226	0·006	0·90—0·95
	Total .	578	191	769	0·011	0·90

The fit between observed and expected ratios is very close and suggests a mono-factorial difference between narrow and broad glumes. The F_3 generation grown from all the F_2 plants in series A shows the following results :—

TABLE XXIII

F_3 behaviour for the inheritance of outer glumes

No. of families studied	Nature of F_2 phenotype	Genotypic constitution	F_3 behaviour	No. of families	
				Observed	Expected
203	Narrow-glumed	NN and Nn	Pure narrow Segregating (3 : 1)	68·0 135·0	67·7 135·4
74	Broad-glumed	nn	Pure broad	74·0	74·0

It will be observed that the fit between observed and expected frequencies is very close. The character is a clear-cut one and naturally there is no difficulty in separating the broad-glumed phenotype from the narrow-glumed one. A single factor, therefore, is responsible for the inheritance of this character and broad glumes appear to be recessive to narrow glumes. The segregating F_3 families gave the frequencies detailed in Table XXIV and the complete data confirms the F_2 theory.

TABLE XXIV

Behaviour of segregating F_3 families for the inheritance of outer glumes

Culture No.	Observed frequency		Total No. of plants	χ^2	P between	Culture No.	Observed frequency		Total No. of plants	χ^2	P between
	Narrow	Broad					Narrow	Broad			
3	48	10	58	1·863	0·10–0·20	78	49	16	65	0·004	0·90–0·95
4	39	16	55	0·491	0·30–0·50	79	33	10	43	0·069	0·70–0·80
5	26	14	40	2·131	0·10–0·20	80	44	18	62	0·537	0·30–0·50
9	35	20	55	3·788	0·05–0·10	81	59	19	78	0·017	0·80–0·90
12	28	9	37	0·009	0·90–0·95	84	45	14	59	0·051	0·80–0·90
15	56	24	80	1·067	0·30–0·50	85	66	23	89	0·033	0·80–0·90
22	31	11	42	0·032	0·80–0·90	87	27	11	38	0·316	0·50–0·70
24	42	9	51	1·471	0·20–0·30	89	51	20	71	0·381	0·50–0·70
32	54	14	68	0·705	0·30–0·50	91	38	15	53	0·308	0·50–0·70
33	59	16	75	0·552	0·30–0·50	93	52	16	68	0·079	0·70–0·80
38	57	12	69	2·131	0·10–0·20	97	35	8	43	0·937	0·30–0·50
39	60	12	72	2·667	0·10–0·20	99	44	19	63	0·894	0·30–0·50
41	51	18	69	0·044	0·80–0·90	102	49	21	70	0·933	0·30–0·50
43	66	20	86	0·140	0·70–0·80	104	29	10	39	0·008	0·90–0·95
46	54	18	72	0	1·0	105	51	18	69	0·044	0·80–0·90
47	68	22	90	0·015	0·90–0·95	108	45	13	58	0·207	0·50–0·70
50	33	10	43	0·069	0·70–0·80	111	46	19	65	0·620	0·30–0·50
51	6	1	7	0·428	0·50–0·70	113	49	15	64	0·083	0·70–0·80
52	35	11	46	0·029	0·80–0·90	115	56	21	77	0·212	0·50–0·70
53	42	19	61	1·229	0·20–0·30	116	52	16	68	0·079	0·70–0·80
55	44	18	62	0·537	0·30–0·50	117	37	12	49	0·007	0·90–0·95
56	50	21	71	0·793	0·30–0·50	118	63	19	82	0·147	0·70–0·80
57	37	12	49	0·007	0·90–0·95	120	38	8	46	1·420	0·20 0·30
59	43	14	57	0·005	0·90–0·95	121	45	17	62	0·194	0·50–0·70
60	38	13	51	0·007	0·90–0·95	124	41	15	56	0·095	0·70–0·80
62	51	15	66	0·183	0·50–0·70	125	51	18	69	0·044	0·80–0·90
63	81	22	103	0·728	0·30–0·50	126	49	11	60	1·423	0·20–0·30
65	39	17	56	0·857	0·30–0·50	129	43	14	57	0·005	0·90–0·95
66	42	17	59	0·458	0·30–0·50	130	39	13	52	0	1·0
69	28	13	41	0·951	0·30–0·50	131	34	17	51	1·889	0·10–0·20
71	63	19	82	0·147	0·70–0·80	132	35	6	41	2·349	0·10–0·20
73	65	19	84	0·253	0·50–0·70	133	53	21	74	0·451	0·50–0·70
74	47	10	57	1·691	0·10–0·20	134	44	14	58	0·023	0·80–0·90
75	74	28	102	0·327	0·50–0·70	135	57	16	73	0·315	0·50–0·70
16	45	14	59	0·051	0·80–0·90	137	42	19	61	1·229	0·20–0·30

TABLE XXIV—*concld.*

Behaviour of segregating F_3 families for the inheritance of outer glumes—concld.

Culture No.	Observed frequency		Total No. of plants	χ^2	P between	Culture No.	Observed frequency		Total No. of plants	χ^2	P between
	Narrow	Broad					Narrow	Broad			
140	49	14	63	0·259	0·50–0·70	207	39	18	57	1·316	0·20–0·30
143	36	7	43	1·744	0·10–0·20	208	48	12	60	0·800	0·30–0·50
144	46	19	65	0·620	0·30–0·50	212	51	17	68	0	1·0
145	48	8	56	3·428	0·05–0·10	215	60	23	83	0·324	0·50–0·70
145A	37	14	51	0·164	0·10–0·20	217	49	19	68	0·313	0·50–0·70
147	36	11	47	0·064	0·80–0·90	220	54	14	68	0·705	0·30–0·50
148	38	18	56	1·524	0·20–0·30	221	49	15	64	0·083	0·70–0·80
150	48	14	62	0·194	0·50–0·70	222	42	13	55	0·055	0·80–0·90
151	38	12	50	0·027	0·80–0·90	224	46	10	56	1·524	0·20–0·30
156	44	12	56	0·381	0·50–0·70	229	46	15	61	0·005	0·90–0·95
157	46	17	63	0·132	0·70–0·80	232	51	11	62	1·741	0·10–0·20
159	59	13	72	1·852	0·10–0·20	233	36	8	44	1·091	0·20–0·30
163	38	9	47	0·859	0·30–0·50	237	54	13	67	1·137	0·20–0·30
165	44	15	59	0·005	0·90–0·95	238	38	14	52	0·103	0·70–0·80
168	32	10	42	0·032	0·80–0·90	240	53	16	69	0·121	0·70–0·80
170	36	17	53	1·415	0·20–0·30	242	36	12	48	0	1·0
171	54	16	70	0·172	0·50–0·70	243	43	20	63	1·529	0·20–0·30
177	42	14	56	0	1·0	245	49	17	66	0·020	0·80–0·90
180	55	20	75	0·110	0·70–0·80	247	37	15	52	0·411	0·50–0·70
182	49	16	65	0·005	0·90–0·95	249	41	10	51	0·812	0·30–0·50
185	41	10	51	0·812	0·30–0·50	252	18	7	25	0·119	0·70–0·80
186	40	17	57	0·727	0·30–0·50	254	45	13	58	0·207	0·50–0·70
187	49	15	64	0·083	0·70–0·80	255	48	20	68	0·705	0·30–0·50
189	38	11	49	0·171	0·50–0·70	257	46	13	59	0·277	0·50–0·70
191	34	11	45	0·007	0·90–0·95	258	45	19	64	0·750	0·30–0·50
193	43	13	56	0·095	0·70–0·80	263	52	14	66	0·505	0·30–0·50
194	48	14	62	0·194	0·50–0·70	265	35	9	44	0·485	0·30–3·50
196	53	14	67	0·601	0·30–0·50	266	39	19	58	1·863	0·10–0·20
197	52	20	72	0·296	0·50–0·70	268	35	10	45	0·185	0·50–0·70
200	51	18	69	0·044	0·80–0·90	271	31	13	44	0·485	0·30–0·50
204	37	16	53	0·761	0·30–0·50	273	28	9	37	0·009	0·90–0·95
205	46	9	55	2·188	0·10–0·20	274	25	11	36	0·592	0·30–0·50
206	41	13	54	0·025	0·80–0·90						

The fit between observed and expected ratios is quite good in most cases. This is confirmed by Table XXV which shows the observed and expected frequency distributions of the χ^2 values obtained above and Fig. 4 indicates the same graphically.

Table XXV

Observed and expected χ^2 distributions of the F_3 families segregating for the nature of outer glumes

χ^2	Observed	Expected
0	5	1·35
0·0001	0	1·35
0·0006	0	4·05
0·0039	14	6·75
0·0158	17	13·50
0·0642	17	13·50
0·1480	25	27·00
0·4550	30	27·00
1·0740	12	13·50
1·6420	13	13·50
2·7060	2	6·75
3·8410	0	4·05
5·4120	0	1·35
6·6350	0	1·35
Total ..	135	135

Frequencies in end classes have been pooled.

Total $\chi^2 = 8·0642$, *P*—between 0·30 and 0·50.

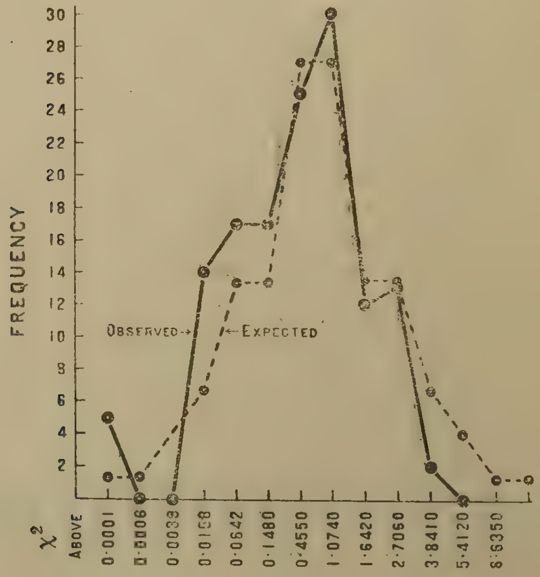


FIG. 4. χ^2 distribution for F_3 families segregating for the nature of outer glumes in barley cross type 21 × type 1.

INTER-RELATIONSHIP OF THE CHARACTERS STUDIED

1. Relation of fertility of the lateral florets to the nature of the outer glumes

Linkage has been observed between the nature of the outer glumes and the fertility of the lateral florets. Type 1 barley possesses broad outer glumes and is two-rowed, whereas type 21 has narrow outer glumes and is six-rowed in fertility.

The mode of segregation in F_2 is shown in Table XXVI given below :—

TABLE XXVI

Fertility and the nature of outer glumes

Series	2-rowed		Intermediate		6-rowed		Total
	Narrow	Broad	Narrow	Broad	Narrow	Broad	
A	26	34	100	34	77	6	277
B	26	34	120	25	58	3	266
C	24	35	90	19	56	2	226
Observed total frequencies . .	76	103	310	78	191	11	769
Expected frequencies on the basis of independent segregation . .	144·18	48·06	288·36	96·12	144·18	48·06	
Expected frequencies on 24·7 per cent cross-over value .	83·24	109·01	312·99	71·51	180·52	11·73	

The fit between observed and expected frequencies on the basis of independent segregation is bad and therefore linkage between the characters studied is apparent. Considering the six-rowed to non-six-rowed fertility and the narrow and broad outer glumes, the cross-over value calculated by the product-ratio method comes to 23·15 per cent.

But as the heterozygous class for fertility (the intermediate class) can easily be separated from the two homozygous classes, the highest efficiency in the estimation of linkage can be reached if the method of maximum likelihood [Fisher, 1932] is employed for the calculation of the linkage value. This, however, involves the formation of six phenotypic classes in place of the usual four and necessitates a modification in the expected probabilities in each phenotypic class. As the maximum likelihood formula for a six-fold classification is not commonly met with, it is proposed to give the calculation of the linkage value by this method in some detail.*

---

*We are indebted to Messrs. J. B. Hutchinson and V. G. Panse of the Institute of Plant Industry, Indore, and Mr. P. V. Krishna Iyer and Dr. S. S. Purewal of the Imperial Institute of Agricultural Research, Pusa, for their kind help in the elucidation of this method.

The observed total frequencies and their expected probabilities are:—

	2-rowed		Intermediate		6-rowed	
—	Narrow	Broad	Narrow	Broad	Narrow	Broad
Observed frequency	76	103	310	78	191	11
Expected probability	$1/4$ $(2\sqrt{\theta}-\ )$	$1/4$ $(1-2\sqrt{\theta}+\theta)$	$1/4$ $(2-2\sqrt{\theta}+2\theta)$	$1/4$ $(2\sqrt{\theta}-2\theta)$	$1/4$ $(1-\theta)$	$1/4\ \theta$

The maximum likelihood in our example can, therefore, be obtained by multiplying each observed class frequency by the logarithm of each probability, summing and equating the whole to zero. Thus—

$$76 \times \log 1/4\,(2\sqrt{\theta}-\theta) + 103 \times \log 1/4\,(1-2\sqrt{\theta}+\theta) + 310 \times \log 1/4\,(2-2\sqrt{\theta}+2\theta) + 78 \times \log 1/4\,(2\sqrt{\theta}-2\theta) + 191 \times \log 1/4\,(1-\theta) + 11 \times \log 1/4\,\theta = 0$$

The calculation may be simplified further by the method suggested and kindly worked out for us by Mr. J. B. Hutchinson, of Indore with the assistance of Mr. V. G. Panse. We take this opportunity of thanking these gentlemen for their ungrudging help and to the former in particular we are grateful for his kind permission to use extracts from his letter No. 1398, dated the 4th April 1936 to the senior author, in order to illustrate the method of calculation. The extracts with slight alterations are given below under quotation marks :

" It is, however, simpler to work with p instead of θ, throughout. Let p = proportion of two-rowed, narrow + six-rowed, broad gametes. Then the expectations in the six classes will be the following, each multiplied by $n/4$:

Expectations in items of p

Glumes	Fertility			Total
	2-rowed	Intermediate	6-rowed	
Natrow . . .	$p\ (2-p)$	$2\ (1-p+p^2)$	$1-p^2$	3
Broad . . .	$(1-p)^2$	$2\ p\ (1-p)$	p^2	1
Total . .	1	2	1	4

Then the expression to be maximised will be the sum of the observed frequencies, each multiplied by the logarithm of the expectation in its class. Thus—

$$76 \log p\ (2-p) + 310 \log 2\ (1-p+p^2) + 191 \log (1-p^2) + 103 \log (1-p)^2 + 78 \log 2p\ (1-p) + 11 \log p^2,$$

which may be simplified by taking advantage of the facts that the logarithm of a product is the sum of the logarithm of its factors, and the logarithm of any constant factor, such as 2, is entirely independent of p and may therefore be ignored. We then get

176 log p + 191 log $(1 + p)$ + 475 log $(1 - p)$ + 76 log $(2 - p)$ + 310 log $(1 - p + p^2)$. This expression will be at a maximum when its first differential is zero. Differentiating with respect to p we get :—

$$\frac{176}{p} + \frac{191}{1+p} - \frac{475}{1-p} - \frac{76}{2-p} - \frac{310\,(1-2p)}{1-p+p^2} = x = 0,$$

an equation which is best solved by trial and error.

The last two trials used were $p = 0{\cdot}246$ and $p = 0{\cdot}247$.

When $p = 0{\cdot}246$ $\quad x = +\,2{\cdot}0929$

When $p = 0{\cdot}247$ $\quad x = -1{\cdot}1467$

To one place of decimals, therefore, the cross-over percentage is **24·7** " and indeed the expected frequencies on the basis of this cross-over value, given at the base of Table XXVI, correspond very closely with the observed total frequencies shown therein.

As shown by Hutchinson [1929] " the variance of p can be estimated with satisfactory accuracy from the rate of change of x for a small change in p near the most likely value of p. When p changed from 0·246 to 0·247 the value of x changed from + 2·0929 to − 1·1467. The change in this case for unit change in p is, therefore, $(2{\cdot}0929 + 1{\cdot}1467) \times 1000 = 3239{\cdot}6 = 1/V\,(p)$

$$\therefore V\,(p) = \frac{1}{3239{\cdot}6} = 0{\cdot}000309$$

and $\sigma(p) = 0{\cdot}0176$ "

It may be mentioned that " the variance of p is obtainable from the equation

$$V(p) = \frac{V(\theta)}{4\theta}$$

Solving for the equation obtained by grouping intermediates with two-rowed barleys (repulsion) gives :—

$\theta = 0{\cdot}0536$

$p = 0{\cdot}231$

$V(\theta) = 0{\cdot}000245$

$V(p) = 0{\cdot}00114$

$\sigma(p) = 0{\cdot}0338$

Similarly solving for the equation obtained by grouping intermediates with six-rowed (coupling) gives :—

$$\begin{aligned} \theta &= 0\cdot5630 \\ p &= 0\cdot740 \text{ (cross-over } = 1-p = 0\cdot260) \\ V(\theta) &= 0\cdot000772 \\ V(p) &= 0\cdot000343 \\ \sigma(p) &= 0\cdot0185 \end{aligned}$$

The inefficiency of the repulsion method is apparent. $V(p)$ in repulsion is 3·69 (or $\frac{0\cdot00114}{0\cdot000309}$) times $V(p)$ for the six-fold classification. On the other hand, much less information is sacrificed by reducing the six-fold to the four-fold classification in the coupling phase, $V(p)$ in coupling being 1·11 (or $\frac{0\cdot000343}{0\cdot000309}$) times $V(p)$ for the six-fold classification. Even so, a loss of eleven per cent in the amount of information is far from negligible."

2. *Relation of maturity to (1) fertility of the lateral florets and (2) nature of outer glumes*

There appears to be no linkage between the maturity of F_2 plants as represented by the number of days taken by them to head out and (1) fertility and (2) nature of outer glumes. This is evident from the similar distributions for maturity shown by the different phenotypes in the F_2 population of series C.

TABLE XXVII

Distribution of F_2 plants according to their maturity and (1) fertility, (2) nature of outer glumes

Character	No. of days taken to head out									Total	Mean	S. Dev.	C. V.
	50	55	60	65	70	75	80	85	90				
FERTILITY													
2-rowed .	1	6	16	25	3	3	2	0	1	57	64·04±0·609	6·82±0·431	10·65±1·063
Intermediate	1	11	47	44	11	8	2	0	0	124	63·45±0·327	5·40±0·231	8·51±0·568
6-rowed .	0	6	11	34	8	5	1	1	1	67	65·50±0·536	6·50±0·379	9·92±1·080
OUTER GLUMES													
Narrow .	0	16	56	82	19	14	3	1	2	193	64·55±0·294	5·05±0·208	9·37±0·442
Broad .	2	8	18	23	3	1	1	0	0	56	62·15±0·487	5·40±0·344	8·69±0·962

The results are obvious and do not require any comments.

3. *Relation of length of ear-heads to (1) fertility of the lateral florets and (2) nature of outer glumes*

Similarly there is no linkage between the length of ear-head and the other two characters studied as is shown below :—

TABLE XXVIII

Distribution of F_2 plants according to their length of ear-heads and (1) fertility, (2) nature of outer glumes

Character	Length of ear-heads in cms.								Total	Means	S. Dev.	C. V.
	5·0	6·0	7·0	8·0	9·0	10·0	11·0	12·0				
FERTILITY												
2-rowed . .	0	3	12	17	14	11	0	0	57	8·34±0·097	1·09±0·069	13·07±0·882
Intermediate .	1	6	17	38	38	18	4	1	123	8·47±0·071	1·17±0·050	13·83±0·945
6-rowed . .	2	4	7	22	20	9	1	0	65	8·31±0·103	1·23±0·073	14·80±1·347
OUTER GLUME												
Narrow . .	2	11	24	52	64	32	5	1	191	8·50±0·060	1·23±0·042	14·47±0·665
Broad . .	1	2	13	21	10	7	0	0	54	8·07±0·104	1·13±0·073	14·00±0·926

The distribution of F_2 plants as regards ear-lengths is similar in all the three types of fertility and also in the two types of outer glumes and suggests independent segregation.

4. *Relation of internode length of ear-heads to (1) fertility of the lateral florets and (2) the nature of outer glumes*

There appears to be no linkage between the internode length of the ear-head and (1) the fertility of the lateral florets and also (2) the nature of outer glumes. The length was measured in cms. by taking the length of ten internodes, measuring always from the third notch from the bottom, with the help of a pair of dividers and scale. Table XXIX shows the frequency distributions of the internode length of the ear-head in relation to other characters under study.

Table XXIX

Distribution of F_2 plants according to their internode length and (1) fertility, (2) the nature of outer glumes

Character	Internode length in cms.							Total	Mean	S. Dev.	C. V.
	2·7	3·0	3·3	3·6	3·9	4·2	4·5				
FERTILITY											
2-rowed	0	11	11	24	10	2	0	58	3·50±0·029	0·324±0·020	9·26±0·734
Intermediate	2	21	27	47	20	1	3	121	3·50±0·024	0·399±0·017	11·40±0·663
6-rowed	2	11	8	36	8	0	2	67	3·50±0·028	0·345±0·020	9·86±1·041
OUTER GLUMES											
Narrow	3	27	35	84	32	3	5	189	3·53±0·017	0·345±0·012	9·77±0·588
Broad	1	16	11	23	5	0	0	56	3·38±0·028	0·306±0·019	9·05±0·611

The above figures clearly show that there is no linkage between the quantitative and the qualitative characters studied.

Summary and conclusions

In a cross between Pusa type 21 and Pusa type 1 which are six-rowed and two-rowed barleys respectively, single factors have been found to determine the inheritance of the following characters :—

(1) Fertility of the lateral florets—two-rowed : intermediate : six-rowed as 1 : 2 : 1.

(2) Awns on the inner glumes of the lateral florets—1 *Nil* : 2 Intermediate : 1 Full.

(3) Nature of outer glumes—3 Narrows : 1 Broad.

The inheritance in items (1) and (2) seems to depend upon the interaction of the same genetic factors and there is complete linkage between—

Two-rowed fertility and development of no awns on the lateral florets,

Intermediate fertility and development of pointed or tipped awns on the lateral florets, and

Six-rowed fertility and development of fully-developed awns on the lateral florets.

Linkage is exhibited when the relationship between fertility and the nature of outer glumes or between the development of awns on the inner glumes of the lateral florets and the nature of outer glumes are studied. The cross-over value in both cases works out to 24·70 per cent.

There is no linkage between fertility or nature of outer glumes and (1) maturity as measured by the number of days taken by individual plants to head out, (2) length of ear-heads in cm. and (3) internode length as represented by the length of ten internodes in cm.

REFERENCES

Albrecht, Kuno (1908). *Landw. Jahr.* **37**, 617-672.

Brady, J. (1934). *Jour. Agric. Sci.* **24**, 209-231.

Bhide, R. K. and Bhalerao, S. G. (1925). *Mem. Dept. Agric. Ind.* (*Bot. Ser.*) **14**, No. 7.

Biffen, R. H. (1905-06). *Jour. Agric. Sci.* **1**, 250-57.

———————(1907). *Jour. Agric. Sci.* **2**, 183-206.

Biffen, R. H. and Engledow, F. L. (1926). *Mins. Agric. Res. Mono.* **4**.

Bose, R. D. (1931). *Ind. Jour. Agric. Sci.* **1**, 58-89.

Bose, R. D. and Dixit, P. D. (1931). *Ind. Jour. Agric. Sci.* **1**, 90-108.

Clark, E. R. and Wilson, H. K. (1933). *Jour. Amer. Soc. Agron.* **25**, 561-72.

Davy, Humphry (1821). " Elements of Agricultural Chemistry ". Third ed., 53-55.

Draghetti, A. (1921). *Staz. Sper. Agric. Ital.* **54**, 145-80.

Davidson, J. and Phillips, M. (1930). *Science* **72**, 401.

Engledow, F. L. (1921). *Jour. Agric. Sci.* **11**, 159-96.

Fisher, R. A. (1932). " Statistical Methods for Research Workers ", 5th edition, p. 279. Oliver and Boyd, London.

Florett, V. H. (1929). *Jour. Amer. Soc. Agron.* **21**, 725-31.

Gaines, E. F. (1917). *Wash. Agric. Expt. Stn. Bull.* **135**.

Garber, R. J. and Olson, P. J. (1919). *Jour. Amer. Soc. Agron.* **7**, 173-86.

Gillis, A. (1926). *Jour. Agric. Res.* **32**, 367-90.

Haberlandt, G. (1914). " Physiological Plant Anatomy", p. 178 : Macmillan & Co., London.

Harlan, H. V. and Hayes, H. K. (1919). *Min. Agric. Expt. Stn. Bull.* **182**, 45-56.

—————————————— (1920). *Jour. Agric. Res.* **19**, 575-91.

Hor, K. S. (1924). *Genetics* **9**, 151-80.

Howard, A. and Howard, G. L. C. (1912). *Mem. Dept. Agric. Ind.* (*Bot. Ser.*) **5**, 1-46.

Huber, A. (1929). *Bibl. Genet.* **19**, 172.

Hunter, H. (1919). *Jour. Dept. Agric. and Tech. Instrn.* (Ireland) **19**, 139-59.

Hutchinson, J. B. (1929). *Genetics* **14**, 519-537.

Kraus, C. (1908). 'Die Lagerung der Getreide ' (The lodging of cereals), Stuttgart, 1908.

—————— (1912). *Beitr. Pflanzenz.* No. **2**, 14-31.

Lienau, D. (1903). Inaug. Diss. Univ. Konigsberg, Vol. 91.

Molden Hawer, K. (1914). *Ztschr. Larder. versuchon astirr.* **17**, No. 12, 886-91.

Nilsson-Ehle, H. (1930). *Sverig. Utsadesforen Tidskr.* **40**, 7-8.

Ramiah, K. and Dharmalingam, S. (1934). *Ind. Jour. Agric. Sci.* **4**, 880-94.

Schieman, E. (1921). *Z. Indukt. Abstamm.-U.-Vererb Lehre.* **27**, 114-33

Schribaux, E. (1920). *C. R. Acad. d' Agric.* **6**, 113-22.

Shaw, F. J. F. and Bose, R. D. (1933). *Ind. Jour. Agric. Sci.* **3**, 771-807.

Thaer, A. D. (1848). " The Principles of Agriculture ". pp. 408-17.

Tabbs, F. R. (1930). *Ann. Bot.* **44**, 147-60.

Tschermak, F. (1923). *Von. Fruwirth's Handb. Landw. Pflanzenz.* **4**, 182.

Ubisch, G. (1916). *Zeits. indukt. Abstam. u. Vererb. Lehre.* **17**, 120-152.

————(1919). *Ibid.* **20**, 65-117.

Vageler, P. (1906). *Jur. Landw.* **54**, 1-30.

Veideman, M. (1927). *Bull. Appl. Bot. Genet. and Plant Breed.* **17**, 1-67. (Ref. taken from Barley Breeding Bibliography of 'The Imperial Bureau of Plant Genetics, Cambridge, England.')

Welton, F. A. (1928). *Bot. Gaz.* **85**, 121-51.

Wexelsen, H. (1934). *Hereditas* **18**, 307-348.

Willis, M. A. (1925). *Jour. Amer. Soc. Agron.* **17**, 334-35.

Barley Breeding Bibliograhy of the Imperial Bureau of Plant Genetics. Cambridge, 1931.

STUDIES IN THE WATER RELATIONS OF RICE

I. EFFECT OF WATERING ON THE RATE OF GROWTH AND YIELD OF FOUR VARIETIES OF RICE

BY

P. K. SEN, M.Sc. (Cal.), Ph.D. (Lond.), D.I.C.

Department of Botany, Calcutta University

(Received for publication on 9th April 1936)

(With Plate IV and seven text-figures)

Introduction

Rice is a crop which is grown under very wet conditions and at some stages of its growth it is partially submerged. Briggs and Shantz [1914], however, found that the water requirements of this plant based on dry matter were almost similar to those of rye. Experiments with some varieties of rice at the Dacca Agricultural Farm, Bengal [1925-26], showed that the average transpiration ratio of this plant was about the same as that found by the former workers. The tradition that very wet conditions are necessary for the growth of rice seems to need investigation.

In India, particularly in Bengal and Assam, rice is cultivated under a variety of field conditions. At one end are fields which are several feet under water during the growing period of the plant, and at the other are fields situated at higher levels in areas where rainfall is comparatively low and irrigation a necessity, and there are many intermediate conditions between these extremes. For the breeding and selection of types to suit these conditions a clear understanding of the nature of the response of the plant to various water conditions seems to be essential.

It is the practice in some parts of Bihar and Orissa, and other irrigated areas, to drain the rice fields at certain stages of the crop and to replace the standing water by fresh water. The practice appears to be justified by experiments made by Sherrard [1920] of the Bihar Agricultural Department. It is therefore of interest to find out the scientific basis of the practice as on it depends the expenditure on irrigation and assessment of water-tax.

According to observations made in culture experiments on rice by the Agricultural Department of Bengal, water standing in the field during certain stages of growth after transplanting suppresses tillering in rice. Where conditions for tillering are favourable the plant continues to produce tillers but the later ones are not generally available for harvest. They either die before reaching maturity or remain green (especially in the early-ripening varieties) when the older tillers are already mature and ready for harvest. In this connection a study of the interrelation of the level of water in the field and the stage of growth of the plant becomes interesting.

It is considered, therefore, that it would be advantageous to elucidate the water relations of the rice plant under different conditions, and to determine its optimum water requirements and the critical stages of watering, and also to investigate the interrelation of watering and the major nutrient factors.

Work in the above directions has been started in the Botany Laboratory of the Calcutta University by the help of a special grant made by the Board of Trustees of the Sir Rash Behari Ghose Endowment and the Calcutta University for the purpose. The result of an experiment on the effects of watering on the rate of growth and yield per plant of four varieties of rice largely cultivated over wide areas in Bengal are presented in this paper. While it may be too early to draw any conclusions regarding water control in the field it is thought that the indications provided by these results will be of interest to those engaged in the problems of rice cultivation.

EXPERIMENTAL PROCEDURE

The investigation was conducted in the experimental area of the Botany Department of the Calcutta University. A wide range of watering was employed during the first seven weeks after transplanting when the production of tillers generally reaches the maximum.

A. Watering scheme

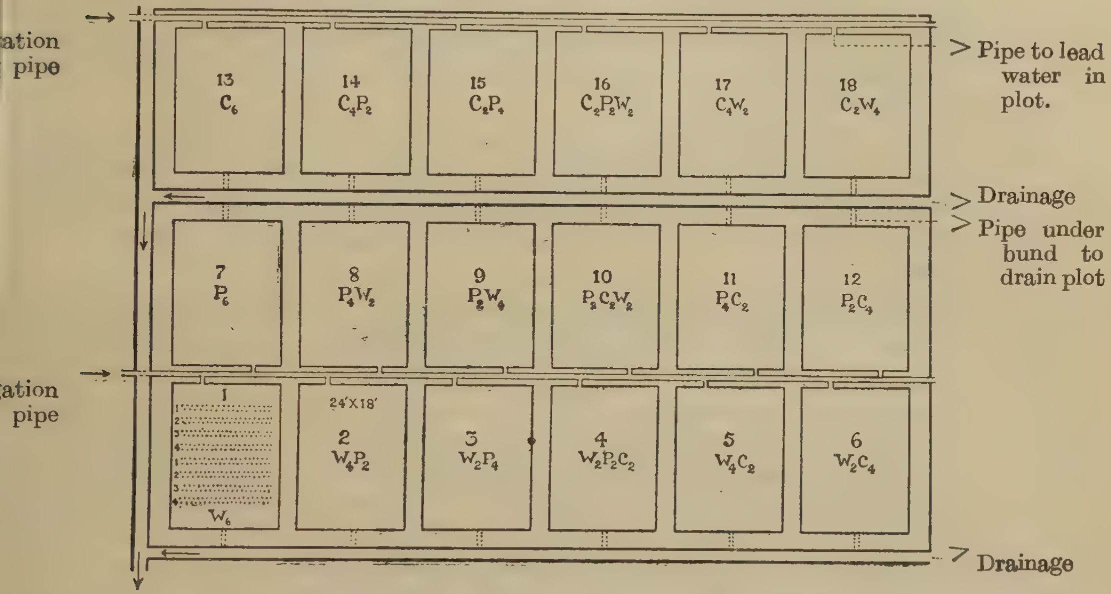
Three inches depth of water, wet but not flooded and cracked condition of field (Table I), were the three conditions of watering used for the investigation. Their effects were studied singly and in combination with the stages of growth of the plant.

TABLE I

Levels of watering

1.	'W'	Field under water; approximately 3 in. depth of water maintained in field.
2.	'P'	Wet field; so watered that there was no water standing in the field nor was any crack allowed to develop.
3.	'C'	Cracked field; drier field with cracks here and there. Uniformity of soil-moisture maintained by eye-judgment.

During the first week after transplanting about an inch of water was kept standing in all plots to enable the seedlings to settle in their new habitat. The next six weeks were divided into three 'two-week' periods so as to represent three stages of growth of the plant. Eighteen treatments combining the different conditions of watering and the different stages of growth of the crop, as shown in Fig. 1, were employed.


ation
pipe
13
C6
14
C4P2
15
C2P4
16
C2P2W2
17
C4W2
18
C2W4
> Pipe to lead water in plot.
> Drainage
> Pipe under bund to drain plot
7
P6
8
P4W2
9
P2W4
10
P2C2W2
11
P4C2
12
P2C4
ation
pipe
1
W6
24'X18'
2
W4P2
3
W2P4
4
W2P2C2
5
W4C2
6
W2C4
Drainage

1. Showing the lay-out of experimental plots and the scheme of watering during the second to the seventh week after transplanting. The letters W, P & C represent the varying condition of watering as per Table I, and the figures after the letters represent the consecutive weeks for which these conditions were maintained in plots during the period of treatment, *e. g.* $W_2 P_2 C_2$ means field under water for first two weeks, wet for the next two weeks and the following two weeks under cracked condition. Plot No I shows the scheme of transplanting in double rows and the arrangement of the varieties 1, 2, 3 and 4, *viz. Kataktara, Bhasamanik, Bhutmuri* and *Jhanji*, respectively.

B. *Lay-out and management of experimental plots*

The experimental area was equipped with proper irrigation and efficient drainage arrangements, so that such supply of water by pumping from the pond in the compound or draining out of excess water from the plots as might be necessary to maintain the condition of treatments could be readily carried out. The area also received a light dressing of cow-dung manure and was thoroughly ploughed before it was divided into eighteen plots, 24 ft. × 18 ft. in size (Fig. 1) and finally prepared for transplanting.

(*i*) *Seeds and seedlings.*—Four pure lines of the following varieties of rice were chosen. Of these *Kataktara, Bhutmuri* and *Jhanji* were early-ripening and the fourth *Bhasamanik* (*Amon*) (Chinaura No. 2) was a late-ripening type. The seeds * were graded for uniformity of size and colour by eye-judgment, sterilized in 0·02 formalin for four hours, dried, and sown in replicated seed-beds on June 4, 1934. Germination commenced from 7th June, 1934 and was nearly complete in all varieties in five days. The order of germination was *Bhutmuri*, *Bhasamanik*, *Jhanji* and *Kataktara*.

(*ii*) *Scheme of transplantation.*—On July 12th, *i.e.* thirty-eight days after sowing, the seedlings were transplanted to the experimental plots. They were selected for uniformity of size and any seedling showing a tiller was rejected. Transplanting in all plots was done on the same day and completed within three hours.

The seedlings were transplanted singly in double rows each nine inches apart, the plants in one line alternating with those of the other. There were eight such rows in each plot and the varieties were planted as shown in plot I in Fig. 1. Between two rows 18 in. space was left as a path, to provide an easy access to the plants for taking growth notes. There were eighty-two plants of each variety in one plot. Owing to the cost of maintaining experimental conditions and want of certain other facilities it was not possible to replicate the plots while all these treatments were considered desirable as a preliminary trial. Thus the rows were replicated in the same plot and the experiment conducted on a single-plant basis.

(*iii*) *Management of the plots.*—After the period of watering, *i.e.* seven weeks after transplanting, the plots which ended with flooded or wet field condition were kept wet until the crop began to ripen, and those ending with treatment 'C' were kept in the same condition during the remaining period of the experiment. In spite of the very efficient draining arrangements the cracked plots were almost saturated twice in the month of August due to heavy rainfall. The experimental condition could, however, be regained in about two days on both occasions.

For a period of about two weeks before harvesting the *Aus*, the field had to be kept dry which was not quite suitable for the *Amon* variety. As soon as the *Aus* paddy was harvested the normal conditions for the experiment were restored in the plots.

C. Growth analysis and yield record

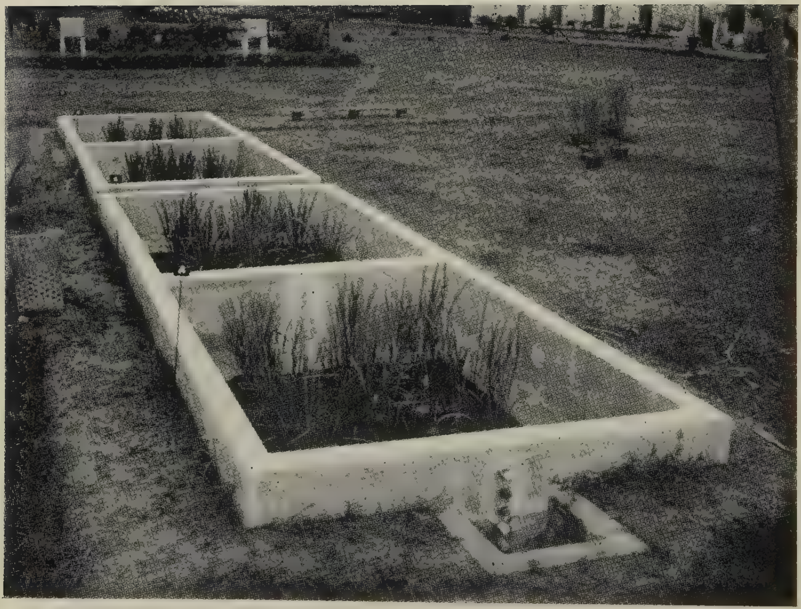
Twenty-five plants of each variety in each plot, using every third plant in one line, were selected as a sample for growth analysis and yield record.

A weekly tiller count was taken from the 24th July to the 11th September, *i.e.* from the end of the second week to the ninth week after transplanting.

Ear-emergence note was taken in the *Aus* varieties on the 4th and 11th September. A final count of tiller and ear numbers was made at harvest.

---

* Seeds of all the varieties were obtained from the Chinsura Rice Research Station of the Department of Agriculture, Bengal, for which thanks are due to the authorities.



General view of the cultures in the cemented tanks.

The *Aus* varieties were harvested early in October. *Kataktara* was ready one week earlier than the two other varieties. The *Amon* paddy was harvested in the third week of November. The plants were very carefully uprooted from the soil and the crown was cleared by removing the soil and the attached roots.

The height of the plant was determined in *Kataktara*, *Bhutmuri* and *Bhasamanik* by measuring the length of the longest tiller of the plant from the base to the tip including the ear. The ears were separated from the shoots at the node at their base and their length measured. The total length of ears in a plant was divided by the number of its ears for average ear length.

Straw, ears and grain were collected separately, dried to constant weight at 98°±1°C. and their weight determined. Dry weight of a single grain was determined as an average of one thousand grains taken at random from the yield of all the twenty-five sample plants.

The results are expressed on a single-plant basis. Standard error is calculated for tiller number at the maximum point and at harvest, height of plant, average length of ears, dry weights of total top, straw and grain.

D. Pot-culture experiment

A pot-culture experiment under watering controlled in suitably constructed cemented tanks was also conducted at the same time. Here three of the above varieties of rice and the four water conditions shown in Table II were used.

TABLE II

Water conditions maintained in tanks

1.	'A'	No water in tank, pots watered to maintain a certain condition of soil-moisture.
2.	'B'	Water in tank up to the level of soil in pots.
3.	'C'	Water in tank up to 3 in. above soil level in pots.
4.	'D'	Water in tank up to 6 in. above the level of soil in pots.

The experimental tanks and the arrangement of pots in them are shown in Plate IV. The tanks were 7 ft. × 10 ft., 2 ft. deep, one foot below ground level and one foot above. They were provided with stoppered outlets at bottom to drain them out and down one of the sides there were outlets so that by running out water the desired level could conveniently be maintained in them. The tanks were watered from the pond.

Garden soil and 10 in. × 10 in. earthen-ware pots were used in this experiment. Seedlings from the same seed-beds were collected as above and transplanted one in each pot. Ten replicates of each variety were taken for each treatment. For the first week after transplanting the pots were kept under about an inch of water in all tanks to enable the seedlings to settle in them. The plants were brought under experimental conditions from the second week. The effect of watering was studied here as a single factor. The four experimental conditions were maintained from the second to the seventh week after transplanting. Seven weeks after transplanting the water level in tanks 'C' and 'D' was gradually lowered so that by the end of the eighth week the plants in these tanks were in 'B' condition. With the setting in of ripening the plants were brought under drier conditions.

Growth analysis was made in this experiment in greater detail and the plants were sampled six weeks after transplanting and again at harvest. The effect of watering on the uptake of nitrogen by the plant was also studied.

A full account of this experiment will appear as a second part of this work. Some results will, however, be discussed in this paper in their bearing on the results of the field experiment.

EXPERIMENTAL RESULTS

Watering treatments used in this experiment resulted in large differences in growth rate and final yield as may be seen from data presented below :—

A. Tillering

Tiller-count data are presented in Tables III to VI in the appendix.

Under the three single treatments W_6, P_6 and C_6, the rate of tillering in the beginning is greater under 3 in. water, *i.e.*, W_6, than under either wet, P_6, or cracked, C_6, condition of the field irrespective of the varieties used for the experiment. By the sixth week after transplanting this rate is surpassed under C_6 in the varieties used except in *Bhutmuri* in which the rate of tillering is higher in water than in the two other conditions, throughout the period of nine weeks after transplanting. In *Kataktara, Jhanji* and *Bhasamanik* the tiller number in C_6 reaches the maximum in these three treatments. An estimate of standard error, however, does not confirm this in *Kataktara*. Wet conditions of the field, P_6, induced larger productions of tillers than the water condition, W_6, only in *Bhasamanik*. In the *Aus* varieties the rate of tillering under wet conditions maintained a level somewhat below that under W_6 during the first nine weeks after transplanting. With the maturation of the shoots there was a second crop of tillers from the base of the *Aus* paddy so that at the time of the harvest these plants had in addition to mature tillers with ripe ears a number of young tillers of various sizes and stages of growth up to the production of ears. This made counting tiller numbers at harvest time difficult in the *Aus* varieties. In spite of the difficulty, however, a count was made in *Kataktara* and the tillers which bore ripened ears were included in the count. In the late ripening variety, *Bhasamanik* (*Amon*), there were no green shoots in the plant at harvest time and a tiller count was made without difficulty. In *Kataktara* (Table III) where the plants were harvested only twelve weeks after transplanting there is an indication of continued tiller production under wet field conditions. Under cracked condition a rapid fall after the maximum and a second rise at harvest indicates that during the later part of the life of the plants new tillers are continuously produced but fail to survive for long. The plants under W_6 in *Kataktara* show no secondary rise in tiller number. In *Bhasamanik* (Table VI) tiller numbers fall very rapidly at harvest under P_6 and C_6. In C_6 the number fell from a maximum of 19·9 to 13·8 (31 per cent) and in P_6 from 19·8 to 13·7 (31 per cent) while in W_6 the fall was from a maximum of 17·8 to 15·0 or 16 per cent only, so that the number of tillers available at harvest was highest at W_6.

Watering differently at different stages of the crop also had a marked effect on tillering. Water for the first two weeks of treatment and wet or cracked condition of the field later (W_2P_4 or W_2C_4) supplied the best condition for tiller growth irrespective of varieties. W_2P_4 maintained the highest level at harvest time. Wet or cracked condition of the field for the first two weeks with flooding later, on the other hand suppressed tillering very considerably and in all varieties the level was below that in any of the three conditions W_6, P_6 and C_6. In none of the varieties except *Kataktara* did flooding for the first four weeks of treatment followed by wet or cracked condition of the field later (W_4P_2 or W_4C_2) show any improvement over W_6, P_6 or C_6. In *Bhasamanik* tillering was reduced under W_4C_2 and in *Jhanji* under W_4P_2 while both of these delayed draining treatments reduced tillering in *Bhutmuri*. In *Kataktara* these treatments showed slight, but not significant, increase in tillering.

The effect of treatments P_4W_2 and C_4W_2 on tillering is similar to those of P_2W_4 and C_2W_4 bringing out clearly that water standing in the field after the first two or three weeks of transplanting suppresses tillering in rice.

In $W_2P_2C_2$ where the crop was in 3 in. water for the first two weeks of treatment and later in wet and cracked condition of field successively, tillering was not much affected ; in *Jhanji* (Table V), however, tiller number reached a maximum higher than that under W_6 and P_6 conditions. In $P_2C_2W_2$ and $C_2P_2W_2$ where the crop was in water during the later stages tillering was very much reduced.

Of the treatments P_4C_2, P_2C_4, C_4P_2 and C_2P_4, only under P_4C_2, *i.e.* where the plants were in wet condition for the first four weeks of treatment and in cracked condition of the field later did tiller number reach a slightly higher maximum than W_6 and P_6 in all varieties. Under P_2C_4, C_2P_4 and C_4P_2 tillers were slightly suppressed, the suppression being comparatively more in P_2C_4 than in the other two conditions.

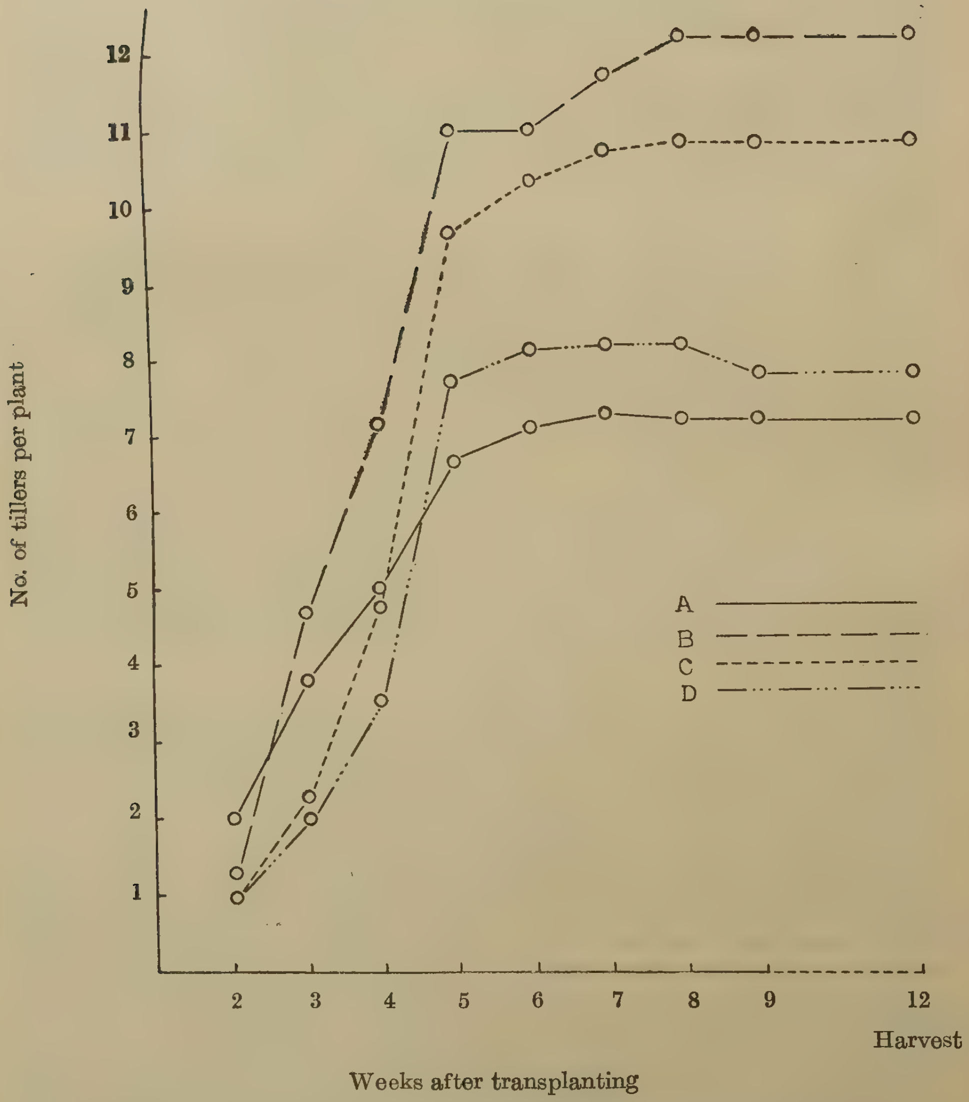


Fig. 2. Effect of varying water condition in pot-culture experiment on tillerin in *Kataktara*.

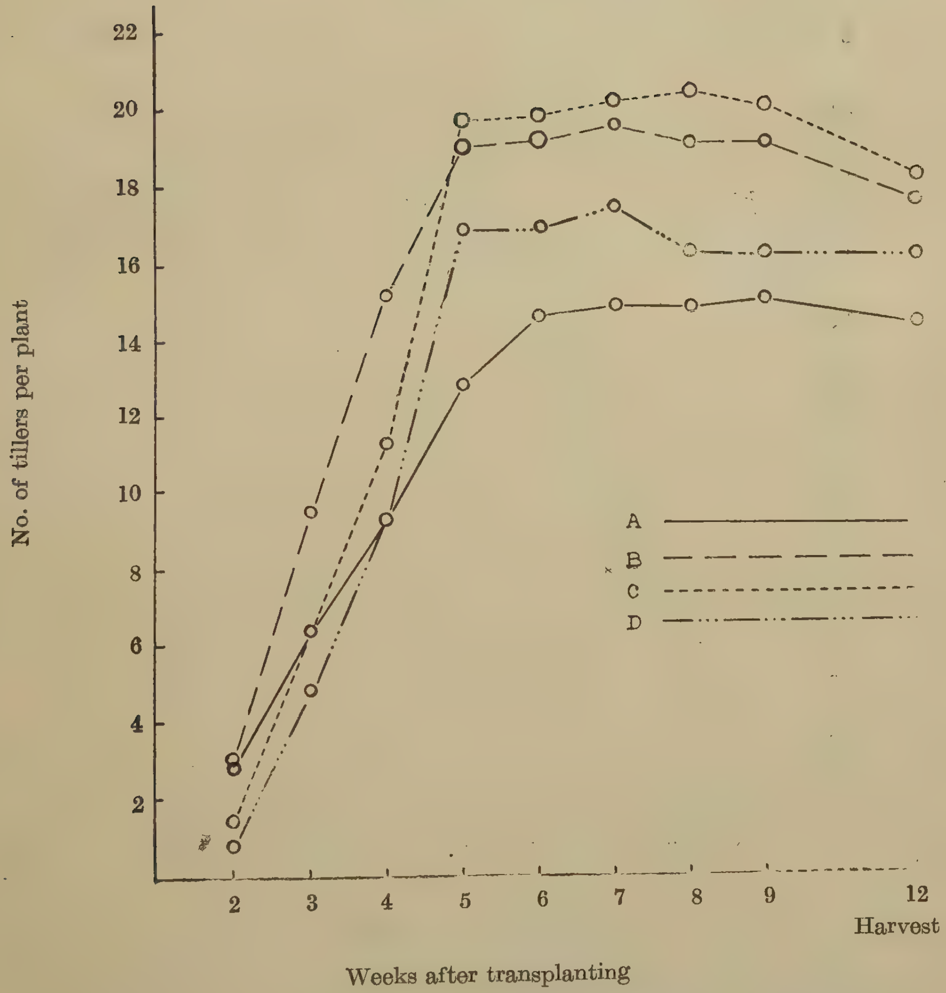


Fig. 3. Effect of varying water condition in pot-culture experiment on tillering in *Bhutmuri*.

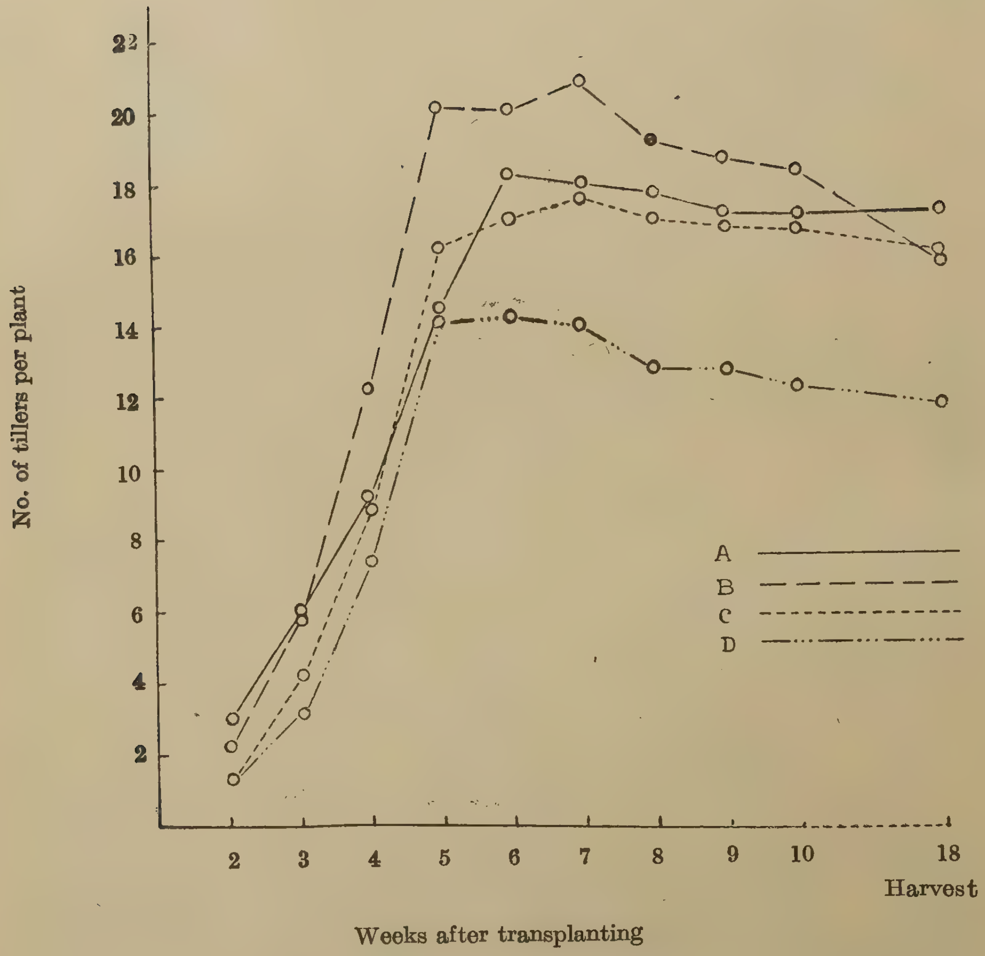


Fig. 4. Effect of varying water condition in pot-culture experiment on tillering in *Bhasamanik*.

The results of the experiment in tanks in pot-culture are shown in Figs. 2 to 4. In the *Aus* varieties *Kataktara* and *Bhutmuri* tiller numbers are minimum under treatment ' A '. *Kataktara* has produced the maximum number of tillers under ' B '. Under ' C ' and ' D ' tiller number in this variety has fallen with increasing depth of water. In *Bhutmuri* the maximum is obtained under ' C ', which corresponds with the response of this variety to W_6 in the field as compared with C_6 or P_6. With further increase in water under ' D ' tillering is reduced.

In the *Amon* variety, *Bhasamanik*, a larger number of tillers is produced under ' B ', but with increasing depth of water tiller number falls. Tillering in ' A ' is less than that under ' B ' but more than that under either ' C ' or ' D '. At harvest the maximum number of tillers is, however, recorded under ' A ', while a considerable number of tillers produced under ' B ' died immaturely.

B. Height of the crop

The effect of watering treatments on the height of the crop was also very marked. The height data are given in Appendix, Table VII. A comparison of the C_6, P_6 and W_6 values shows how the height of the plant increases with increase in water. This is confirmed in the pot-culture experiment where a continued increase in the height of the plant from ' A ' to ' D ' resulted.

The effects of watering differently at different stages of the crop also bring out clearly the dwarfing effect of the cracked condition on the plant in the field.

C. Ear-emergence

Ear-emergence note was taken twice in the field in the case of the *Aus* paddy on 4th September 1934 and 11th September 1934 and a count of ears at harvest was made in *Kataktara* and *Bhasamanik*. The data are presented in the Appendix in Table VIII. Cracked condition of the field delayed flowering in the *Aus* varieties and the process was hastened under increasing moisture condition of the soil. These observations confirm the findings of the Department of Agriculture, Bengal [1923-24]. In the pot-cultures, however, flowering was hastened under condition ' A '. The contrary result in the field may be related to the degree of drought in a cracked field. Also some mechanical injury to the root system of the plant caused by the cracking of the field may influence the rate of its growth and development.

Number of ears at harvest as percentage of the maximum number of tillers noted in a plant during the first nine weeks after transplanting was different in the two types of paddy, *viz. Kataktara* (*Aus*) and *Bhasamanik* (*Amon*). In *Kataktara* the percentage was higher under wet or cracked condition than in water.

The plants which were in 3 in. water in the early stages after transplanting and in drier field condition later on, produced a greater percentage of ears than those which were under water or wet condition during the later weeks of treatment.

In *Bhasamanik* the number of ears as a percentage of the maximum number of tillers in a plant was higher where the crop was in water particularly during the later period of treatments.

The average length of ears was also affected by watering treatments. The results are given in Table IX in the Appendix. In *Kataktara* (*Aus*) longer ears were produced under W_2P_4, W_2C_4 and C_2P_4. Water in the field during the later stages of growth tended to reduce the length of ears in *Kataktara*.

In *Bhasamanik*, on the other hand, longer ears were produced under conditions in which the crop was in water for the greater part of the period of watering treatment. The highest value was obtained under P_2W_4 in this variety.

D. Yield of straw and grain

The watering treatments used resulted in marked differences in the total yield of straw and grain per plant, as well as in their distribution. The harvest data* are given in Table X in the Appendix and the results are diagrammatically shown in Figs. 5 to 7. The dry weight of the total top, *i.e.*, straw and grain together, reached the maximum for all varieties, *Aus* and *Amon*, under W_2P_4, *i.e.*, where the crop was in 3 in. water for the earlier stages of growth and in wet field for the later period. Under W_2C_4 this value in *Kataktara* appears to be affected very little while in *Bhutmuri* and *Bhasamanik* it reached only about eighty-one per cent and sixty-six per cent respectively of that under W_2P_4. Under W_6 total dry weight of the top reached 78·2 per cent, 89·5 per cent and 95·6 per cent of that under W_2P_4 in *Kataktara*, *Bhutmuri* and *Bhasamanik*, respectively, the last showing no significant difference. The corresponding values are 92·8 per cent, 75·5 per cent and 75·3 per cent under P_6 and 74·8 per cent, 60·5 per cent and 47·1 per cent under C_6.

---

* In *Jhanji* the grains only were estimated.

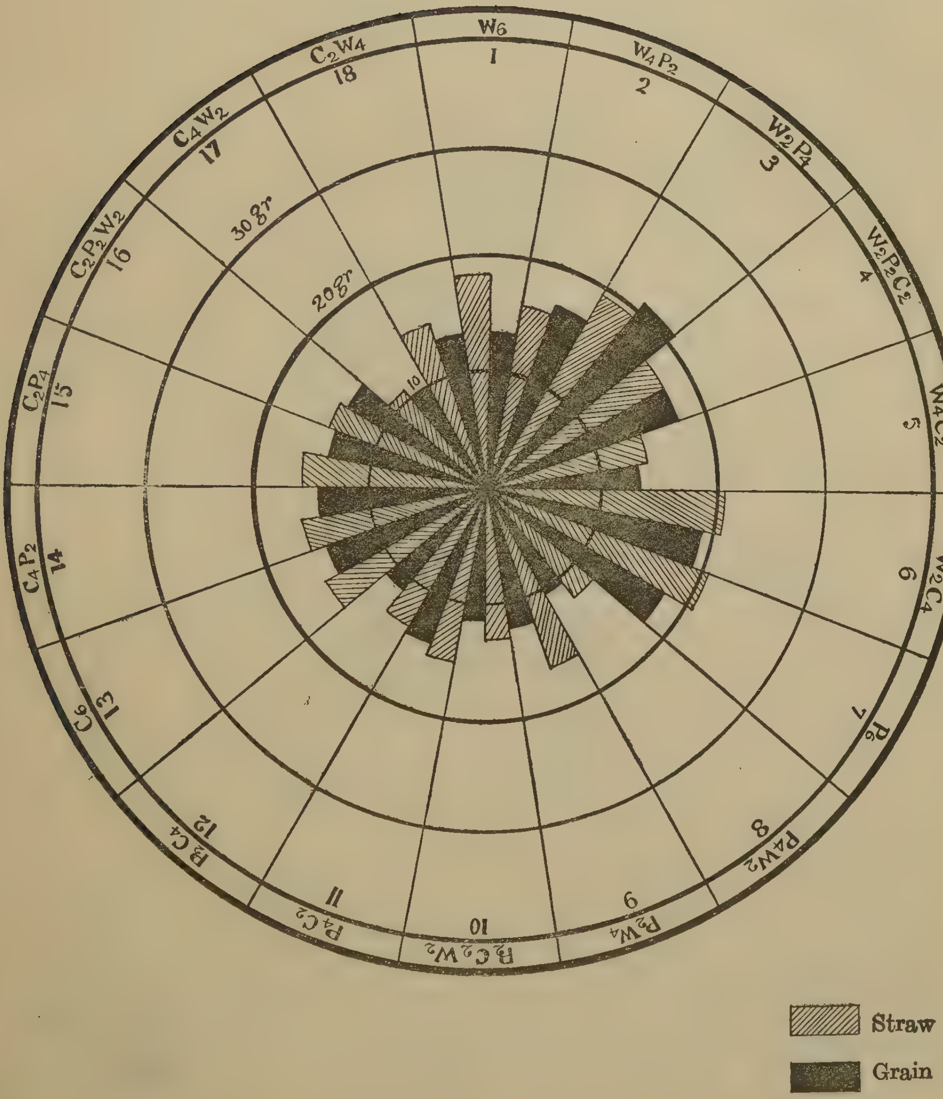


Fig. 5. Effect of varying water condition in field on yield in *Kataktara*

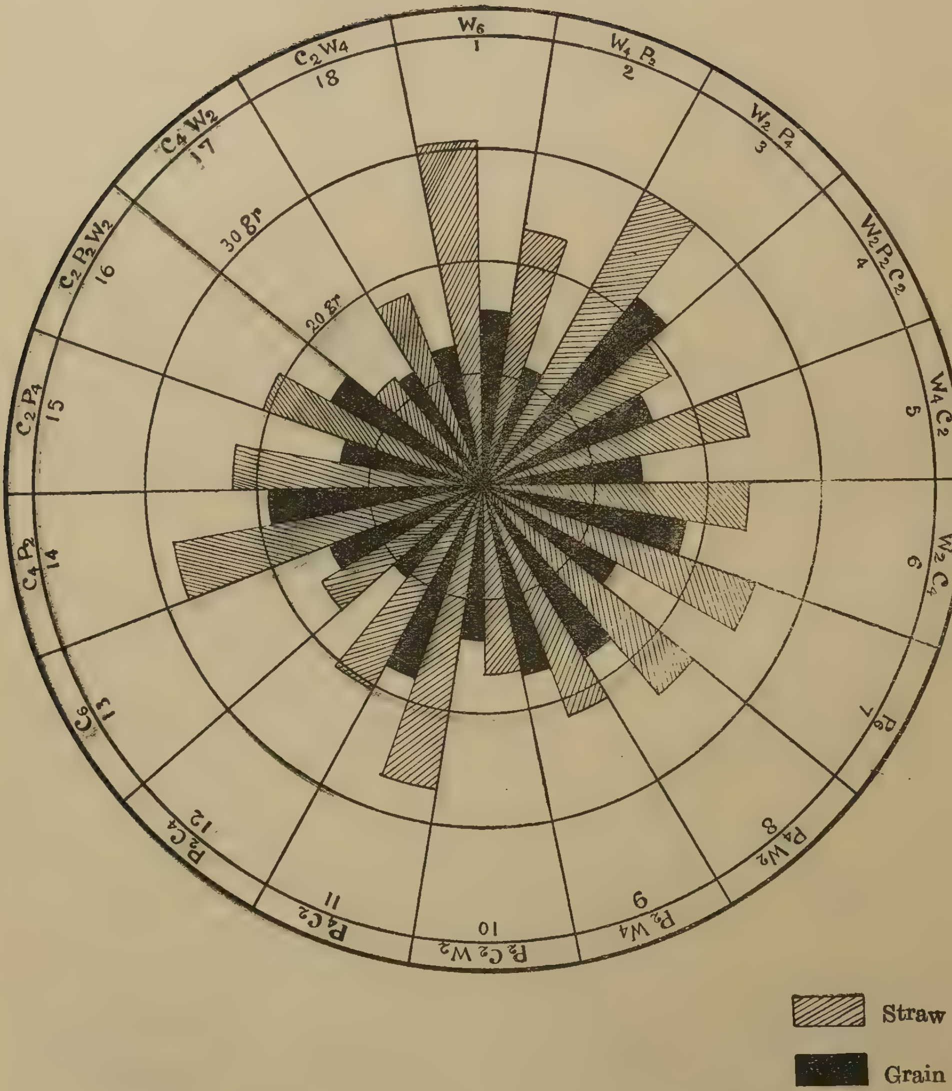


Fig. 6. Effect of varying water condition in field on yield in *Bhutmuri*

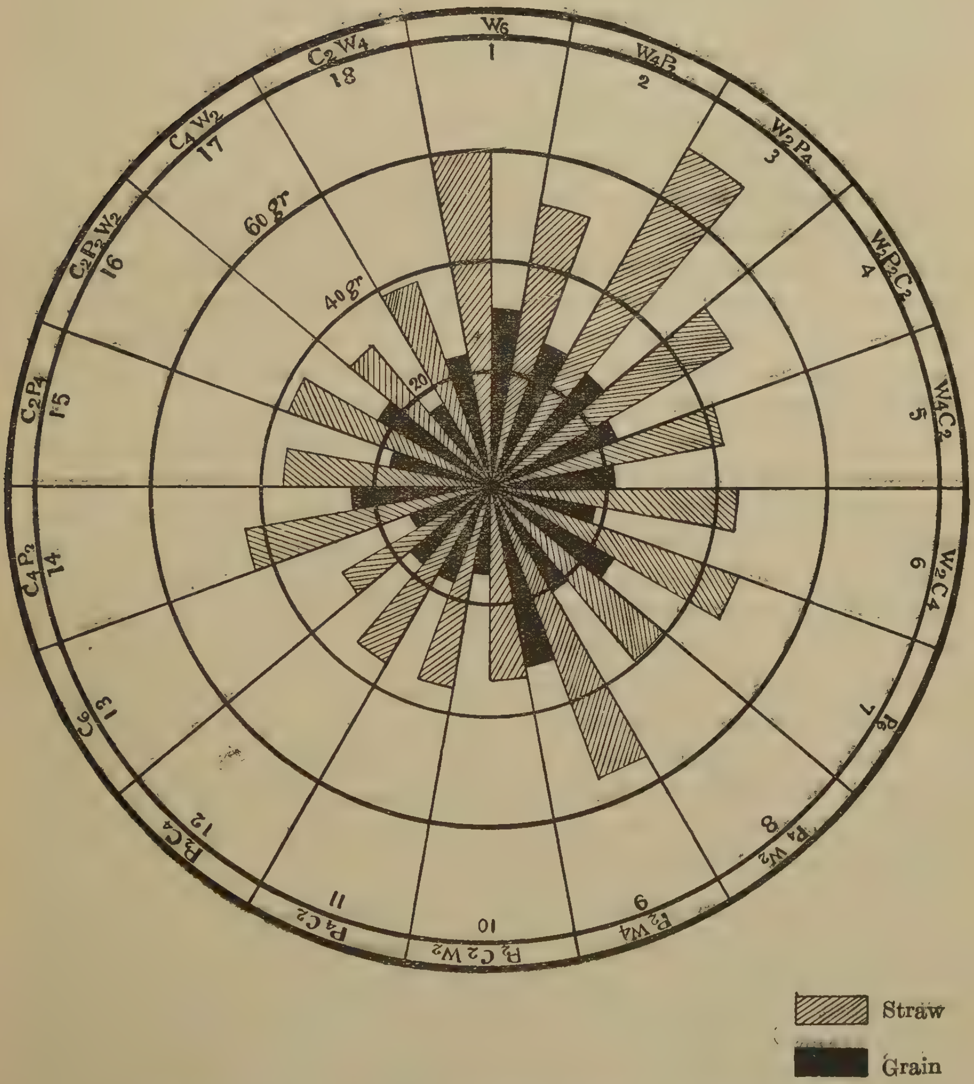


Fig. 7. Effect of varying water condition in field on yield in *Bhasamanik*

Application of water differently at different stages of growth of the plant brings out the fact that water in the field after a period of three weeks after transplanting has a diminishing effect on total yield of straw and grain in *Aus* paddy and that the adverse effect of only wet but not flooded or drier condition of the field during the earlier stages of the crop cannot be got over by flooding it later on. The results of the treatments P_4W_2, C_4W_2, P_2W_4 and C_2W_4 indicate that delayed flooding of the field reduces the total yield of straw and grain.

The effects of the treatments on the ratio of straw to grain was different in *Aus* and *Amon* rice. In all the three varieties of *Aus* paddy yield of grain per plant was highest under W_2P_4. The amount of grain as percentage of straw under this treatment was 108 per cent in *Kataktara*, actually the maximum value of this variety. In *Bhutmuri* also this value is very high. In *Kataktara* and *Bhutmuri* the proportion of straw to grain increases with increasing soil-moisture [Russell, 1932] as may be seen from the values of grain as percentage of straw under C_6, P_6 and W_6; *viz.* 90·9 per cent, 80·5 per cent and 73·9 per cent in *Kataktara* and 87·4 per cent, 53·4 per cent and 49·8 per cent in *Bhutmuri*, respectively (Table X). The net yield of grain per plant under these three conditions, however, is highest in *Kataktara* under P_6 and in *Bhutmuri* under W_6, but the W_6 value in *Bhutmuri* is not significantly different from the C_6 value. Treatments of interrelation of watering and stages of growth of the crop show that field flooded with about three inches depth of water for three weeks after transplanting and only wet afterwards presents the most suitable condition for yield of grain for the three varieties of *Aus* paddy used in this investigation.

Yield of grain per plant in the *Amon* variety of rice, *viz. Bhasamanik*, is highest (32·1 ± 1·302) under W_6, and this value is practically reached (31·6 ± 1·377) under P_2W_4. The proportion of grain to straw is 57·9 per cent in P_2W_4 and 53·9 per cent in W_6. A gradual diminution of the amount of grain in W_4P_2, W_2P_4, $W_2P_2C_2$, W_4C_2 and W_2C_4 (Table X) clearly brings out the limiting effect of water during the period of treatments in this variety.

The values of grain per plant under W_6, P_6 and C_6 are 32·1 ± 1·302 grms., 25·2 ± 1·114 grms. and 15·0 ± 0·835 grms. respectively and the corresponding values for the percentage of grain to straw are 53·9 per cent, 53·8 per cent and 49·5 per cent. The amount of grain per plant under P_2W_4, P_4W_2, C_2W_4, and $C_2P_2W_2$ (Table X) indicates that the adverse effect of limiting water during the early weeks could, however, be made good by flooding the field during the period of six to seven weeks after transplantation, the degree of recovery depending on the period for which, and the degree to which, the crop is subjected to limiting water.

The weight of a single grain under the watering treatments is also presented in Table X, in the Appendix. Water in the field for the whole of the period of treatment or during the later stages of growth tends to decrease the single grain weight in *Kataktara*. Higher values in this variety are obtained under P_6, C_4P_2 and C_2P_4 while comparative drought does not seem to affect the quality. But in all the other varieties the dry weight of a single grain decreases with drought [Russell, 1932] the values under W_6, P_6 and C_6 are 20·2 mg., 20·0 mg. and 18·8 mg. for *Bhutmuri*, 21·1 mg., 20·5 mg. and 20·3 mg. for *Jhanji* and 16·9 mg., 15·3 mg. and 15·4 mg. for *Bhasamanik*, respectively. The highest values in *Jhanji* and *Bhasamanik* were obtained under P_2W_4 and for *Bhutmuri* under C_2W_4 where the values for *Jhanji* and *Bhasamanik* are also higher than those under W_6. Single-grain weight in *Bhutmuri* has very high values in almost all treatments of 3 in. water in the field for the whole or part of the growing period.

DISCUSSION AND AGRICULTURAL BEARING OF RESULTS

Standing water in the field during the first two or three weeks after transplanting seems to be beneficial for the growth of tillers in rice. Later on, however, a field with sufficient moisture but no water standing in it favours tillering while standing water definitely suppresses it.

In the field experiment, tillering in the early stages was greater in plants which were flooded with about 3 in. depth of water. Draining the field three weeks after transplanting was an improvement in the condition for tillering.

Tillering of the crop during its early stages, may not be directly affected by flooding. For in the experiment in pot-culture water standing above the level of soil suppressed tillering throughout the growing period of the plant. Here the number of tillers per plant increased when the plants were immersed in water up to the level of the soil as compared with the pots in dry tank, as well as with those immersed in greater depths of water. This indicates that with increase in the water level tillering increases up to a point beyond which it falls again, and that standing water above the soil level appears to have no beneficial effect on tiller production. Only in one *Aus* variety, *Bhutmuri*, maximum number of tillers were obtained under 3 in. of water but the number was reduced when the water level was increased to 6 in. Even in this variety, however, tiller production was greatest up to the fourth week after transplanting in pots which were in water up to the soil level only (Fig. 3). The effect in field may be possibly due to the production and maintenance of conditions of temperature and texture of the soil suitable for the plant immediately after transplanting.

During the later stages, when the plant has established itself, draining the field brings about an improvement in the condition for tiller-growth indicating better aeration of the growing parts at the base and also of the soil. At this stage a cracked field has actually led to the production of larger number of tillers all of which, however, do not survive owing to the limiting effect of soil moisture. Wet but not flooded condition of the field after it has been under two to three inches of water for about three weeks after transplanting appears to provide a better condition for the growth and development of tillers.

Flowering in *Aus* (early-ripening) rice under cracked condition of fields may be delayed because of the retardation of the rate of growth by the limiting effect of soil moisture [Russell, 1932]. Also some mechanical injury on the root-system of the crop caused by the cracking of the field may influence its growth which it cannot get over during the shorter growing period.

In the *Aus* varieties maximum yield was obtained under conditions most favourable for growth and development of tillers, *i.e.*, about three inches of water in the field for the first two or three weeks after transplanting and wet condition of the field later on up to the time of complete development (6-7 weeks) including the reproductive parts.

Excess of moisture during the later stages of growth reduces the proportion of grain to straw [Russell, 1932].

In the *Amon* variety, *Bhasamanik*, where the period of growth and development is much longer (about 15 weeks) than in the *Aus*, a suitable level of water should be maintained longer in the field so that soil-moisture is not limiting during floral development. The yield of grain in this variety was higher under conditions in which standing water was maintained in the field up to the seventh week after transplanting while the total dry weight of straw and grain together reached maximum under conditions where the field was under about three inches of water for three weeks after transplanting and only wet later on. The water requirements of this variety in the field appear to be generally similar to those of the *Aus* varieties, with the difference that standing water has to be maintained in the field for a longer time for the *Amon*, corresponding to its longer maturation period.

These results appear to show the significance of the practice of draining fields at certain stages of the crop in some irrigated areas referred to in the introduction. A certain depth of water in the field during the early stages of growth is most favourable for the seedlings to settle down, the draining promotes growth and development of tillers, whereas the fresh supply again submerges the lower parts of the plant and suppresses the growth of tillers which may not be mature in time, and supplies the soil moisture necessary for the flowering stages.

The above results show that different varieties of rice have considerable differences in their water requirements. Of the *Aus* varieties used in this experiment *Kataktara* requires the least quantity of water and *Bhutmuri* the most.

The results further show that in all varieties there is ample scope for economy if irrigation is arranged according to the requirements of the plant. A field-scale experiment on similar lines will be advantageous to elucidate further the problems under investigation.

SUMMARY

Three *Aus* and one *Amon* varieties of rice largely cultivated over wide areas in Bengal were grown under different water conditions.

Three inches depth of water, wet but not flooded and cracked condition of the field were the three different water conditions employed ; their effects were studied singly and in combination with three different stages of growth of the crop. Second and third weeks, fourth and fifth weeks, and sixth and seventh weeks after transplanting represented three different stages used. In all, eighteen treatments were employed.

The effect of partial submergence on the growth of the plant was also studied in pot-culture in cemented tanks.

Standing water suppresses tiller production in rice, the number of tillers decreasing with increase in the depth of water.

Height of the plant increases as water increases.

Flowering in the *Aus* varieties in the field is delayed under cracked field condition.

Number of ears as percentage of maximum number of tillers in a plant and the length of ears vary with the different treatments and also vary for the same treatment as between *Aus* and *Amon* rice.

Optimum of watering for maximum yield is different in different varieties.

Flooding the field after transplanting for about three weeks in *Aus* and somewhat longer in *Amon* corresponding with its longer period of growth, followed by subsequent de-watering is beneficial to the plant. Standing water does not seem to affect the plant directly. It is suggested that water produces and maintains a certain texture and temperature condition of the soil suitable for young seedlings durin their earlier stages of growth. With the later stages of growth draining is necessary for aeration.

In conclusion the author wishes to express his grateful thanks to Professor S. P. Agharkar, M.A., Ph.D., F.L.S., Head of the Department of Botany, Calcutta University, for facilities offered and for his interest during the progress of this investigation.

REFERENCES

Annual Report of the Dept. of Agric., Bengal (1923-24), Page ii.

———————————— (1925-26), Page ii.

Briggs, J. Lyman and Shantz, H. L. (1914). *Jour. Agric. Res.* **3**, 1.

Russell, E. J. (1932). " Soil conditions and plant growth, " Sixth Edition, Longmans Green & Co., Page 45.

Sherrard, G. C. (1920). *Agric. Res. Inst. Pusa, Bulletin* No. **96**, 20.

P. S.—Since the preparation of this manuscript, B. N. Singh and others have published one paper on ' Investigations into the Water Requirement of Crop Plants ' *in the Proceedings of the Indian Academy of Sciences*, Vol. 1, March, 1935. Average water requirement of rice on dry-weight basis found by these workers is about the same as recorded by Briggs and Shantz and the Department of Agriculture, Bengal. They further report varietal differences in the water requirement of rice. Differential effect of watering on rice varieties is also described in this paper.

Appendix

TABLE III

Effect of different watering treatments on tillering in Kataktara (Aus)

Watering		Tillers per plant								
	Date	24-7-34	31-7-34	7-8-34	14-8-34	21-8-34	28-8-34	4-9-34	11-9-34	At harvest
	Weeks after trans-planting	2	3	4	5	6	7	8	9	12
1 W_6 .		1·32	3·28	5·56	5·84	6·36	6·64	7·00±0·163	6·72	6·64±0·167
2 W_4P_2 .		1·44	2·92	4·96	5·88	7·04	7·60±0·361	7·28	6·76	6·72±0·187
3 W_2P_4 .		1·48	3·28	5·96	6·76	7·84	7·80	8·12±0·343	7·84	7·88±0·219
4 $W_2P_2C_2$.		1·16	2·56	4·08	5·28	6·96±0·248	6·84	6·84	6·64	7·48±0·283
5 W_4C_2 .		1·40	3·04	5·08	6·04	6·84	7·28±0·376	6·36	6·24	5·90±0·324
6 W_2C_4 .		1·28	3·08	4·92	6·00	7·68±0·382	7·04	7·20	7·16	7·56±0·295
7 P_6 . .		1·00	3·00	4·28	5·40	6·44	6·36	6·56	6·78±0·228	7·76±0·260
8 P_4W_2 .		1·12	2·96	4·04	5·48	5·60	5·72	6·08±0·258	6·04	6·00±0·178
9 P_2W_4 .		1·04	2·92	4·56	5·20	5·64	6·08±0·223	6·00	6·04	6·00±0·200
10 $P_2C_2W_2$.		1·12	2·24	3·04	4·64	5·52	5·56	5·48	5·80±0·316	5·60±0·245
11 P_4C		1·20	2·68	4·08	5·40	7·44	7·64	7·84±0·377	6·76	7·24±0·226
12 P_2C_4 .		1·12	2·08	3·76	4·08	5·68±0·256	5·32	5·16	5·00	6·68±0·287
13 C_6 . .		1·00	2·20	4·08	5·08	6·76	6·68	7·58±0·254	6·40	6·92±0·299
14 C_4P_2 .		1·16	2·80	4·60	5·20	6·16	6·48±0·278	6·16	5·88	6·84±0·256
15 C_2P_4 .		1·28	2·48	5·00	5·64	5·84	6·52±0·259	5·60	4·88	6·16±0·236
16 $C_2P_2W_2$.		1·08	2·60	5·36	5·44	5·44	5·72±0·249	5·68	5·72	5·68±0·180
17 C_4W_2 .		1·08	2·64	4·28	5·16	5·40	5·80±0·231	5·50	5·52	5·44±0·154
18 C_2W_4 .		1·20	2·72	5·16	5·24	5·58	5·72±0·242	5·68	5·68	5·64±0·187

TABLE IV

Effect of different watering treatments on tillering in Bhutmuri (Aus)

	Watering		Tillers per plant							
		Date	24-7-34	31-7-34	7-8-34	14-8-34	21-8-34	28-8-34	4-9-34	11-9-34
		Weeks after trans-planting	2	3	4	5	6	7	8	9
1	W_6 .		3·60	8·52	12·2	15·6	16·5 ±0·477	15·8	16·0	15·8
2	W_4P_2 .		3·68	8·16	11·8	12·8	12·6	13·5 ±0·679	12·9	11·8
3	W_2P_4 .		3·16	7·68	12·4	15·5	17·0	17·5 ±0·592	17·3	16·7
4	$W_2P_2C_2$		3·12	6·96	10·4	12·0	15·2	14·7	15·7 ±0·675	15·5
5	W_4C_2 .		3·80	8·12	11·9	13·0	12·9	13·4 ±0·536	12·7	12·3
6	W_2C_4 .		3·08	7·76	11·7	13·9	17·4	17·9 ±0·474	17·0	16·9
7	P_6 .		3·36	7·48	11·2	12·6	15·0	16·0 ±0·412	15·4	14·6
8	P_4W_2 .		3·08	7·20	12·0	12·9	13·2 ±0·455	12·1	11·4	11·4
9	P_2W_4 .		3·24	6·92	11·2	12·3	13·1	13·4 ±0·688	13·4	13·2
10	$P_2C_2W_2$		2·88	5·52	7·44	10·2	12·6	13·6 ±0·697	12·8	13·0
11	P_4C_2 .		3·04	7·16	12·1	13·0	17·2 ±0·656	17·0	16·0	16·2
12	P_2C_4 .		3·16	7·40	10·4	11·3	14·4 ±0·608	13·6	13·1	12·0
13	C_6 .		2·76	6·16	9·88	11·6	14·9	15·8 ±0·493	15·6	14·3
14	C_4P_2 .		3·12	7·40	9·72	11·9	13·4	14·0 ±0·465	13·6	12·8
15	C_2P_4 .		2·96	6·28	11·0	13·0	13·4	14·5 ±0·432	13·4	12·8
16	$C_2P_2W_2$		3·04	6·28	10·5	12·0	12·3 ±0·435	12·0	11·6	12·3
17	C_4W_2 .		2·36	6·08	9·60	11·4	12·5	12·8 ±0·443	10·8	10·8
18	C_2W_4 .		3·28	7·32	11·1	11·3	12·5 ±0·523	12·0	11·3	11·0

TABLE V

Effect of different watering treatments on tillering in Jhanji (Aus)

Watering	Tillers per plant								
	Date	24-7-34	31-7-34	7-8-34	14-8-34	21-8-34	28-8-34	4-9-34	11-9-34
	Weeks after trans-planting	2	3	4	5	6	7	8	9
1 W_6 .		3·16	7·08	10·4	13·5	15·4	16·4±0·748	15·0	15·1
2 W_4P_2 .		3·20	6·64	10·2	10·8	13·7±0·632	13·3	13·0	13·0
3 W_2P_4 .		3·36	7·96	11·8	14·5	17·2	17·4	17·8±0·804	15·9
4 $W_2P_2C_2$		3·44	7·56	11·5	13·8	18·6±0·733	18·3	17·4	16·6
5 W_4C_2 .		3·20	6·48	9·92	11·0	13·5	14·0	15·4±0·781	15·0
6 W_2C_4 .		3·40	8·08	11·0	13·8	20·0	20·5±0·583	19·7	18·0
7 P_6 .		2·76	5·92	8·64	12·6	13·9	16·1±0·530	14·9	13·8
8 P_4W_2 .		2·96	6·06	9·00	12·9	14·3	15·3±0·492	14·8	12·6
9 P_2W_4 .		3·20	7·20	10·1	11·8	12·0	13·6	13·9±0·469	13·3
10 $P_2C_2W_2$		2·76	5·24	7·44	9·84	12·8±0·535	12·5	12·1	12·8
11 P_4C_2 .		2·88	6·24	9·12	13·1	16·7	19·0±0·779	15·9	14·7
12 P_2C_4 .		2·88	6·00	8·00	9·12	13·0±0·679	12·0	12·3	11·0
13 C_6 .		3·08	6·16	10·3	12·6	17·7±0·699	17·2	17·2	16·8
14 C_4P_2 .		3·20	6·52	9·92	11·9	14·5	15·1±0·575	13·6	13·4
15 C_2P_4 .		2·84	6·28	9·52	12·8	13·8	14·4±0·700	13·7	12·4
16 $C_2P_2W_2$		2·88	6·40	9·76	10·4	11·0	11·8±0·594	11·8	11·0
17 C_4W_2 .		2·96	6·04	10·1	12·2	13·8±0·432	13·6	12·5	10·8
18 C_2W_4 .		3·16	6·80	10·4	10·6	11·3	11·6	12·5±0·530	11·2

TABLE VI

Effect of different watering treatments on tillering in Bhasamanik (Amon)

Watering	Tillers per plant									
	Date	24-7-34	31-7-34	7-8-34	14-8-34	21-8-34	28-8-34	4-9-34	11-9-34	At harvest
	Weeks after transplanting	2	3	4	5	6	7	8	9	18
1 W_6		2·96	7·16	12·0	14·6	16·3	17·8±0·693	17·5	17·0	15·0±0·475
2 W_4P_2		2·88	6·96	11·8	14·0	16·7	17·2±0·803	15·3	15·4	12·0±0·544
3 W_2P_4		2·92	6·88	10·5	15·0	20·5±0·817	20·5	18·8	19·0	17·8±0·577
4 $W_2P_2C_2$		2·80	6·44	10·3	13·2	17·2	18·4±0·571	17·9	17·7	13·8±0·336
5 W_4C_2		2·52	6·76	11·4	14·3	15·2	15·5±0·184	14·8	14·4	11·4±0·379
6 W_2C_4		2·72	6·56	10·5	14·8	19·6	20·1	20·8±0·976	20·3	13·9±0·407
7 P_6		2·16	5·88	10·4	16·0	17·4	19·8±0·824	18·9	18·6	13·7±0·298
8 P_4W_2		2·40	6·44	10·2	13·2	13·6	15·0±0·633	14·9	14·6	11·8±0·254
9 P_2W_4		2·76	5·96	10·4	12·0	14·6	14·8±0·408	14·3	14·0	12·3±0·410
10 $P_2C_2W_2$		2·08	4·88	7·68	10·6	14·9	15·0±0·592	14·6	14·0	11·0±0·265
11 P_4C_2		2·32	6·52	11·1	14·1	19·0	20·4±0·851	19·8	18·5	12·8±0·365
12 P_2C_4		2·36	5·36	9·04	12·1	17·1	19·0±0·690	17·1	14·9	12·6±0·356
13 C_6		2·16	4·80	8·60	12·5	18·2	18·8	19·6	19·9±0·616	13·8±0·440
14 C_4P_2		2·08	4·84	8·72	12·7	17·0	19·0±0·810	17·4	16·1	12·7±0·680
15 C_2P_4		2·52	6·16	10·4	14·0	16·4	19·2±0·764	16·6	14·1	11·2±0·311
16 $C_2P_2W_2$		2·24	5·28	9·36	11·8	13·0	14·4±0·432	14·2	13·8	11·7±0·465
17 C_4W_2		2·04	4·60	8·84	12·0	12·4	12·7±0·457	11·9	11·4	11·2±0·356
18 C_2W_4		2·64	5·40	9·52	11·0	13·4	13·5	13·8±0·451	13·1	12·8±0·500

TABLE VII

Effect of different watering treatments on the height of the rice plant, in cm.

	Watering	*Kataktara* (*Aus*)	*Bhutmuri* (*Aus*)	*Bhasamanik* (*Amon*)
1	W_6	130·2±1·448	132·6±1·416	127·3±1·194
2	W_4P_2	131·2±1·328	123·7±1·154	123·6±1·423
3	W_2P_4	131·8±1·227	131·7±1·097	128·5±1·405
4	$W_2P_2C_2$	126·3±2·301	121·4±0·803	118·6±1·340
5	W_4C_2	125·5±2·620	122·5±1·301	118·9±1·713
6	W_2C_4	127·9±1·433	122·2±1·109	111·1±1·680
7	P_6	125·1±1·922	121·1±1·336	115·0±1·235
8	P_4W_2	124·5±1·646	122·8±1·532	114·2±1·464
9	P_2W_4	127·3±1·731	126·8±1·326	131·1±1·541
10	$P_2C_2W_2$	118·4±1·059	122·0±1·425	109·1±1·572
11	P_4C_2	114·8±1·434	125·6±1·928	105·9±1·226
12	P_2C_4	115·2±2·391	116·5±1·226	104·3±1·106
13	C_6	115·7±1·568	110·4±0·997	96·0±1·079
14	C_4P_2	121·0±1·911	117·0±1·824	107·0±1·766
15	C_2P_4	122·5±2·341	120·7±1·257	109·4±1·435
16	$C_2P_2W_2$	124·7±2·178	123·0±2·015	114·3±1·726
17	C_4W_2	116·5±1·508	116·2±1·361	108·5±1·243
18	C_2W_4	125·2±1·393	125·2±1·177	114·6±1·103

TABLE VIII*

Effect of different watering treatments on the production of ears in rice

Serial No.	Watering	Ears per plant									
		Kataktara (Aus)				*Bhutmuri (Aus)*		*Jhanji (Aus)*		*Bhasamanik (Amon)*	
		4-9-34 Weeks after transplanting	11-9-34	4-10-34	No. of mature ears as per cent of maximum No. of tillers 9 weeks after transplanting	4-9-34	11-9-34	4-9-34	11-9-34	17-11-34	No. of mature ears as per cent of maximum No. of tillers
		8	9	Harvest		8	9	8	9	Harvest	
1	W_6	4·48	5·88	6·64	94·9	10·5	14·5	5·70	13·8	15·0	84·3
2	W_4P_2	4·60	6·44	6·72	88·4	8·84	10·3	3·44	7·60	12·0	69·8
3	W_2P_4	4·88	7·08	7·88	97·0	10·2	13·8	4·40	13·8	17·2	83·9
4	$W_2P_2C_2$	3·40	6·08	7·48	107·5	8·60	14·0	2·24	14·0	13·7	74·5
5	W_4C_2	4·44	5·80	5·90	81·0	9·20	11·5	4·92	12·6	11·4	73·5
6	W_2C_4	4·40	6·64	7·56	98·4	9·48	15·4	3·96	16·4	13·8	66·3
7	P_6	3·92	5·16	7·76	114·5	10·5	13·6	3·52	10·6	13·7	69·2
8	P_4W_2	2·16	3·92	6·00	98·7	5·96	10·5	1·60	10·4	11·0	73·3
9	P_2W_4	3·04	5·56	6·00	98·7	8·80	11·7	3·16	12·0	12·2	82·5
10	$P_2C_2W_2$	3·08	4·96	5·60	96·6	5·68	11·1	0·64	10·1	11·0	73·3
11	P_4C_2	4·36	6·24	7·24	92·3	6·24	13·8	0·08	10·9	12·8	63·7
12	P_2C_4	2·12	4·36	6·68	117·6	7·56	11·2	1·84	9·60	12·6	66·3
13	C_6	1·72	4·92	6·92	95·1	1·16	11·6	0·00	5·88	13·7	68·8
14	C_4P_2	4·76	5·64	6·84	105·6	8·48	11·8	4·28	9·84	12·7	66·8
15	C_2P_4	4·00	4·52	6·16	94·5	5·36	11·2	0·64	9·28	11·2	58·3
16	$C_2P_2W_2$	3·52	5·20	5·68	99·3	7·80	10·2	0·44	9·32	11·7	81·3
17	C_4W_2	2·32	4·02	5·44	93·8	2·84	8·36	0·13	7·40	11·1	87·4
18	C_2W_4	3·36	5·24	5·64	98·6	6·96	8·60	1·32	9·20	12·8	92·8

---

* An estimate of error for the ear numbers at harvest in *Kataktara* and *Bhasamanik* may be made from the S. E. of their tiller numbers at harvest. The ear numbers and tiller numbers do not differ in *Kataktara* and in *Bhasamanik*, the difference is very little.

TABLE IX

Effect of different watering treatments on the length of ears in rice, in cm.

	Watering	*Kataktara (Aus)*	*Bhasamanik (Amon)*
1	W_6	23·7±0·214	21·8±0·196
2	W_4P_2	23·8±0·185	20·9±0·244
3	W_2P_4	25·0±0·158	20·2±0·245
4	$W_2P_2C_2$	24·3±0·160	20·3±0·283
5	W_4C_2	23·3±0·200	21·0±0·303
6	W_2C_4	24·7±0·139	19·6±0·198
7	P_6	24·9±0·197	20·1±0·216
8	P_4W_2	23·6±0·212	20·7±0·305
9	P_2W_4	23·9±0·219	22·4±0·194
10	$P_2C_2W_2$	23·1±0·272	19·8±0·218
11	P_4C_2	24·3±0·196	19·0±0·191
12	P_2C_4	23·5±0·179	18·9±0·197
13	C_6	24·2±0·131	18·0±0·187
14	C_4P_2	24·1±0·282	19·8±0·194
15	C_2P_4	24·9±0·219	19·7±0·292
16	$C_2P_2W_2$	23·8±0·278	20·6±0·274
17	C_4W_2	23·3±0·153	18·8±0·211
18	C_2W_4	23·7±0·237	21·3±0·202

TABLE

Effect of different watering

Watering	*Kataktara (Aus)*					*Bhutmuri*		
	Dry wt. of total top (grms.)	Dry wt. of straw (grms.)	Dry wt. of grain (grms.)	Grain as per cent of straw	Dry wt. of single grain (mg.)	Dry wt. of total top (grms.)	Dry wt. of straw (grms.)	Dry wt. of grain (grms.)
1 W_6 .	32·7±1·159	18·8±0·775	13·9±0·457	73·9	16·8	46·0±2·111	30·7±1·293	15·3±0·932
2 W_4P_2	33·7±1·172	16·5±0·665	17·2±0·582	104·2	17·7	34·3±1·813	23·0±1·813	11·3±0·716
3 W_2P_4	41·8±1·708	20·1±0·805	21·7±0·998	108·0	17·5	51·4±2·256	29·8±1·470	21·6±1·062
4 $W_2P_2C_2$	35·9±1·954	17·8±1·040	18·1±1·026	101·7	17·5	35·5±2·070	19·6±1·122	15·9±1·039
5 W_4C_2	28·1±1·227	14·6±0·632	13·5±0·655	92·4	17·4	37·9±1·504	24·1±1·168	13·8±0·551
6 W_2C_4	40·2±2·510	20·8±1·223	19·4±1·202	93·3	17·4	41·8±1·719	23·7±0·911	18·1±0·909
7 P_6 .	38·8±1·629	21·0±0·946	17·8±0·910	80·5	17·5	38·8±1·666	25·3±1·324	13·5±0·811
8 P_4W_2	23·2±0·870	12·8±0·513	10·4±0·471	81·3	16·8	38·2±2·234	20·8±1·357	17·4±0·992
9 P_2W_4	28·6±1·231	16·4±0·796	12·6±0·486	76·3	17·1	38·4±2·005	21·6±1·131	16·8±0·967
10 $P_2C_2W_2$	24·7±1·109	13·2±0·576	11·5±0·646	87·1	17·4	30·4±1·625	16·7±1·031	13·7±0·676
11 P_4C_2 .	32·9±1·096	18·1±0·698	14·8±0·646	81·8	17·0	44·9±2·784	26·9±1·654	18·0±1·237
12 P_2C_4 .	25·8±0·877	14·1±0·526	11·7±0·502	82·9	17·3	31·1±1·754	20·4±1·300	10·7±0·627
13 C_6 .	31·4±1·206	16·4±0·819	14·9±0·475	90·9	17·2	31·1±1·578	16·6±0·845	14·5±0·799
14 C_4P_2 .	31·6±1·240	16·4±0·708	15·1±0·613	92·1	17·6	47·3±2·641	28·1±1·820	19·2±1·055
15 C_2P_4 .	31·0±1·146	16·4±0·696	14·6±0·576	89·0	17·7	34·7±1·603	22·1±1·112	12·6±0·672
16 $C_2P_2W_2$	29·4±0·903	14·8±0·541	14·6±0·464	98·6	17·1	35·7±1·673	20·4±1·109	15·3±0·650
17 C_4W_2	21·5±0·708	10·9±0·358	10·6±0·509	97·2	16·7	23·7±0·795	12·0±0·390	11·7±0·456
18 C_2W_4	29·5±1·006	15·4±0·560	14·0±0·616	90·9	17·4	30·6±1·771	18·0±1·218	12·6±0·717

X

treatment on yield in rice

(Aus)		*Jhanji (Aus)*		*Bhasamanik (Amon)*				
Grain as per cent of straw	Dry wt. single grain (mg.)	Dry wt. of grain (grms.)	Dry wt. of single grain (mg.)	Dry wt. of total top (grms.)	Dry wt. of straw (grms.)	Dry wt. of grain (grms.)	Grain as per cent of straw	Dry wt of single grain (mg.)
49·8	20·2	19·9±1·301	21·1	91·6±2·724	59·5±2·055	32·1±1·302	53·9	15·9
48·5	20·1	16·3±0·928	21·5	78·1±3·685	51·2±2·461	26·9±1·578	52·5	16·5
72·5	20·3	24·2±1·271	21·6	95·8±3·709	70·0±2·370	25·8±1·325	36·9	14·9
81·1	20·1	17·1±1·020	21·3	74·3±3·643	50·4±2·586	23·9±1·165	47·4	15·4
57·3	20·2	14·2±0·815	20·2	63·9±2·872	41·6±2·166	22·3±0·882	53·6	16·4
76·4	20·0	21·9±1·252	21·1	62·8±3·298	43·6±2·601	19·2±0·927	44·0	14·8
53·4	19·9	19·5±0·965	20·5	72·2±3·080	47·0±2·233	25·2±1·114	53·8	15·3
83·5	19·9	18·6±1·014	20·8	60·7±3·212	39·5±2·290	21·2±1·106	53·7	16·0
77·8	20·0	17·0±1·002	22·0	86·1±4·370	54·5±3·272	31·6±1·377	57·9	17·0
82·0	19·9	13·9±0·751	21·0	49·1±1·933	34·0±1·499	15·1±0·716	44·4	15·8
66·9	19·8	16·5±1·077	20·4	54·7±1·716	36·3±1·126	18·4±1·008	50·9	15·6
52·4	19·5	13·3±0·836	20·1	53·8±2·002	35·8±1·361	18·0±0·862	50·3	15·8
87·4	18·8	13·5±0·798	20·3	45·2±2·133	30·2±1·422	15·0±0·835	49·5	15·4
68·3	20·3	16·3±1·033	21·3	67·7±3·345	43·7±1·880	24·0±1·672	54·9	15·4
57·0	19·9	14·6±0·882	20·4	54·7±2·650	36·4±1·565	18·3±1·271	50·3	15·7
75·0	20·2	16·1±0·858	21·0	60·8±2·866	38·2±1·918	22·6±1·465	59·2	15·3
97·5	19·4	11·7±0·734	21·1	49·2±2·269	32·7±1·484	16·5±0·735	50·5	15·7
70·0	20·4	15·6±0·837	21·4	62·6±2·970	38·5±1·874	24·1±1·401	62·6	16·8

HETEROSIS IN RICE

BY

B. S. KADAM

Crop Botanist to the Government of Bombay

G. G. PATIL

AND

V. K. PATANKAR

Assistants in Rice Breeding, Karjat

(Received for publication on 21st September 1936)

INTRODUCTION

The luxuriant vegetative growth of the first generation hybrids is a common feature in many crop plants. The phenomenon was first observed by Koelreuter in 1761 and is known as " hybrid vigour " or " Heterosis ". The exact cause or causes of heterosis are not yet known, and at present it is one of the most controversial subjects in the realm of genetics.

Heterosis is of both practical and academic interest to a plant-breeder. In crop plants such as maize, tomato, brinjals, where it is easy to produce hybrid seed on a mass scale, commercial usage of hybrid vigour is being made to obtain increased yields. In cereals, such as wheat, oats, sorghum and rice, hybrid vigour has been observed, and is of only scientific interest as mass-production of hybrid seed is not possible in these crops, except probably in sorghum. In rice there appears to be only one investigation, although the crop has been extensively handled. It was, therefore, decided to collect data on heterosis from crosses which were made for economic purposes. The results are reported in this paper.

MATERIAL AND METHODS

The data on hybrid vigour were obtained from the following crosses :—

(1) K79 × P6, (2) K184 × K79, (3) K226 × B52, (4) Nagkeshar × Ratanghose and (5) B211 × Sabour-2. All the varieties are pure lines. Except the fourth cross all others were made for economic purposes. The strains K79 and K184 are early selections from the Kolamba variety extensively grown in North Konkan. The variety K226 is a late Kolamba selection. The strains B52 and B211 are selections from the Bhadas variety of South Kolaba. From the Patni variety P6 was isolated at the Ratnagiri Farm, while Sabour-2 is an improved type from Bihar and was kindly supplied by Mr. Mahbub Alam, Rice Specialist, Bihar and Orissa.

Crosses were made in the season of 1932-33 and F_1 plants raised next year. The hybrids were transplanted between the parents on both sides. The distance between the plants was eight inches in rows eighteen inches apart. The first two crosses are early maturing and were grown on an upland (high level land). The other crosses were grown in a low-lying field.

Measurements were taken when the plants were in dough stage. The statistical analysis was made according to Fisher [1932].

RESULTS

Height

The data on height of plants are given in Table I. Ten tillers from each plant were measured and averages obtained. In the first three crosses the F_1 plants are less in height than the taller parent. The differences are from 1·29 to 5·61 per cent and are statistically significant in the last case only. In the fourth and the fifth cross the hybrids are taller than the taller parents from 2 to 2·39 per cent, the differences being not significant. Over the short parents the hybrids differ from 7·48 to 84·04 per cent and in all the cases the differences are significant.

There is no indication of heterosis. Greater height appears to be partially dominant. The variety Ratanghose in the fourth cross is a dwarf. In the F_2 of this cross there was monogenic segregation of tall and dwarf plants. The 84·04 per cent increase in height of the hybrid over the dwarf parent is therefore due to a gene. If this cross is excluded then the increases in heights over the shorter parents are only from 7·48 to 15·04 per cent.

TABLE I

Comparison of mean heights of hybrids and their parents

Cross	No.* of plants	Height in centimeter		Increase or decrease of hybrids over parents in per cent	t	
		Range	Mean		Observed	5 per cent
(I)						
F_1	2	119·62—119·85	119·73±0·113			
♀ K79	5	119·00—131·00	124·30±2·56	—3·68	1·77	2·571
♂ P6	5	101·80—110·70	106·20±1·52	12·74	8·84	
(II)						
F_1	4	127·25—135·11	129·62±1·84			
♀K184	5	105·00—117·00	112·67±2·33	15·04	5·67	2·365
♂ K79	5	125·00—140·00	131·31±2·43	—1·29	0·55	
(III)						
F_1	7	113·00—129·77	122·77±2·23			
K226	5	114·70—118·00	115·92±0·58	5·91	2·97	2·228
♂ B52	5	125·00—137·57	130·07±2·17	—5·61	2·34	
(IV)						
F_1	5	133·12—146·14	137·57±3·45			
♀ Nagkeshar	5	122·00—146·00	134·87±4·70	2·00	0·50	2·306
♂ Ratanghose	5	73·44— 77·60	74·75±0·73	84·04	23·52	
(V)						
F_1	3	124·16—130·33	128·10±1·97			
♀ B211	5	118·33—131·00	125·11±2·05	2·39	1·04	2·447
♂ Sabour-2	5	112·90—126·90	119·18±2·73	7·48	2·64	

Number of culms per plant

In Table II are shown average number of tillers per plant of parents and their hybrids. Out of the five crosses only in the first the hybrids show greater number of tillers than the higher parent, but the difference is not significant. In other crosses the F_1s show from six to thirty-five per cent less tillering than the more tillering parents. Over the less tillering parents the hybrids show statistically significant increases in all except in the third cross. The differences range very abnormally, from very low to very high (from 7 to 289 per cent) over the poor parents.

---

* As the combinations were made primarily for economic purposes only a few flowers were crossed, hence the number of F_1 plants is small. In some cases the number of F_1 plants was further reduced due to failure of some seeds to turn out successful crosses.

The F_1 plants thus do not show any hybrid vigour, as none of them exceeds significantly the higher branching parent.

TABLE II

Comparison between mean tillers of hybrids and their parents

Cross	No. of plants	Culms per plant		Increase or decrease of hybrids over parents in per cent	*t*	
		Range	Mean		Observed	5 per cent
(I)						
F_1	2	8—8	8·00±0·00			
♀ K79	5	3—5	3·40±0·39	35·29	11·5	2·571
♂ P6	5	4—11	6·20±1·23	29·03	1·5	
(II)						
F_1	4	7—9	7·75±0·47			
♀ K184	5	5—12	8·40±1·16	—7·74	0·51	2·365
♂ K79	5	3—7	5·40±0·69	43·52	2·80	
(III)						
F_1	7	6—13	8·43±0·996			
♀ K226	5	7—11	9·00±0·83	—6·33	0·44	2·228
♂ B52	5	6—9	7·20±0·58	17·08	1·07	
(IV)						
F_1	5	5—8	7·00±0·62			
♀ Nagkeshar	5	1—3	1·80±0·37	288·89	7·10	2·306
♂ Ratanghose	5	5—13	10·00±1·41	—30·00	1·94	
(V)						
F_1	3	7—12	9·33±1·45			
♀ B211	5	3—7	5·00±0·89	86·60	2·54	2·447
♂ Sabour-2	5	11—18	14·40±1·28	—35·21	2·61	

Length of panicle

The data on length of panicle are given in Table III. In none of the crosses there is any significant increase in length of panicles of the hybrids over the longer-panicled parent. Over the short-panicled parents the increases of the hybrids are significant in all cases. The sixty-three per cent increase of the hybrid over Ratanghose in cross 4 is due to the dominance of long panicle of Nagkeshar, the F_2 showing a 3 : 1 segregation of long and short panicles respectively.

TABLE III

Comparison between mean length of main panicles of hybrids and their parents

Cross	No. of plants	Panicle length in centimeters		Increase or decrease of hybrids over parents in per cent	*t*	
		Range	Mean		Observed	5 per cent
(I)						
F_1	2	23·82—24·21	24·01±0·19			
♀ K79	5	23·60—26·23	25·10±0·47	—4·34	2·13	2·571
♂ P6	5	21·24—23·23	22·33±0·33	7·52	4·31	
(II)						
F_1	4	23·87—26·37	24·99±0·605			
♀ K184	5	18·21—22·01	19·82±0·61	26·08	6·01	2·365
♂ K79	5	25·03—27·90	26·21±0·48	—4·66	1·58	
(III)						
F_1	7	22·61—25·60	24·01±0·44			
♀ K226	5	21·57—22·78	22·33±0·21	7·52	3·43	2·228
♂ B52	5	22·64—24·50	23·82±0·31	0·80	0·35	
(IV)						
F_1	5	23·30—24·60	23·88±0·22			
Nagkeshar	5	21·00—25·50	23·28±0·92	2·58	0·62	2·306
♂ Ratanghose	5	13·47—15·80	14·59±0·37	63·67	21·60	
(V)						
F_1	3	25·10—25·90	25·40±0·23			
♀ B211	5	22·50—27·20	25·10±0·82	1·19	0·35	2·447
♂ Sabour-2	5	22·03—25·15	23·26±0·57	9·20	3·45	

Yield

The data on yield are given in Table IV. Out of the five crosses only in two, 1 and 4 the average yield of hybrids, 36·41 and 65·12 per cent is significantly better than the higher-yielding parents. In the cross K184 × K79 although the mean yield of F_1s is greater by 39·41 per cent over the higher-yielding parent, the difference is not significant. In the fifth cross the average yield of the F_1s is slightly better than the better parent. In the cross K226 × B52 the mean yield of the F_1s is actually less than the higher-yielding parent, but the difference is not ignificant.

There is therefore no heterosis in three out of the five crosses. The crosses K79 × P6 and Nagkeshar × Ratanghose, however, show definite tendency towards heterosis.

TABLE IV

Comparison between average yields of hybrids and their parents

Cross	No. of plants	Yield in grms.		Increase or decrease of hybrids over parents in per cent	*t*	
		Range	Mean		Observed	5 per cent
(I)						
F_1	2	20·45—23·24	21·84±1·397			
♀ K79	5	7·31—16·44	10·37±1·62	110·61	5·36	2·571
♂ P6	5	12·37—21·82	16·01±1·67	36·41	2·67	
(II)						
F_1	4	20·70—38·63	27·84±3·89			
♀ K184	5	11·65—26·12	19·97±2·75	39·41	1·65	2·365
♂ K79	5	11·90—26·78	18·06±2·52	54·15	2·10	
(III)						
F_1	7	17·28—55·12	33·75±4·59			
♀ K226	5	20·82—30·10	24·52±1·808	37·64	1·87	2·228
♂ B52	5	30·06—37·90	34·41±1·51	—1·92	0·13	
(IV)						
F_1	5	17·65—31·02	27·03±2·39			
♀ Nagkeshar	5	7·36—13·22	9·46±1·18	185·73	6·58	2·306
♂ Ratanghose	5	11·67—21·44	16·37±1·57	65·12	3·71	
(V)						
F_1	3	22·47—42·44	33·54±5·87			
♀ B211	5	11·69—30·86	23·06±3·88	45·45	1·49	2·447
♂ Sabour 2	5	22·35—47·34	30·91±4·52	8·51	0·35	

Weight of whole plant

Fully-matured plants were uprooted and after washing off the soil from roots they were weighed. Unfortunately, due to an oversight weights of the two crosses, K79 × P6 and K184 × K79, were not taken. The data on the remaining three crosses are shown in Table V.

In the K226 × B52 cross the mean weight of hybrids is less than the Bhadas parent.

In the fourth and fifth crosses the F_1 plants show higher average weights than the heavier parents, but the increases are not significant. Only in the Nagkeshar-Ratanghose cross the increase in weight is significant over the low weight parent. The tallness of the Nagkeshar and the higher tillering of the Ratanghose have combined to give greater weight to the hybrid.

The weight of the whole plant is considered as the best criterion of heterosis. In the present crosses there is no evidence of hybrid vigour for this character.

TABLE V

Comparison between average weights of whole plants of hybrids and their parents

Cross	No. of plants	Whole plant weight in grms.		Increase or decrease of hybrids over parents in per cent	*t*	
		Range	Mean		Observed	5 per cent
(III)						
F_1	7	77·96—230·34	144·28±18·91			
♀ K226	5	99·22—152·38	126·15±8·83	14·37	0·89	2·228
♂ B52	5	113·40—201·99	167·26±16·02	—13·74	0·92	
(IV)						
F_1	5	116·94—219·71	175·77±16·94			
♀ Nagkeshar	5	31·89—56·70	46·07±4·33	281·53	7·34	2·306
♂ Ratanghose	5	99·22—184·27	153·79±15·64	14·29	0·95	
(V)						
F_1	3	88·59—173·64	145·29±28·40			
♀ B211	5	31·89—173·64	116·94±29·01	24·24	0·69	2·447
♂ Sabour-2	5	92·76—219·71	143·18±21·54	1·47	0·059	

DISCUSSION

Jones [1926] found from 0·6 to 7·2 centimeters increases in heights of hybrids over the taller parents. He has, however, taken the average of both parents to compare with the respective hybrids. This has made the hybrids appear taller, ranging from 5·9 to 10·9 centimeters. These figures are considered by Jones as indicative of hybrid vigour. We found that out of five, the average height of the F_1 plants is actually less than the taller parents in three cases, while in two, the hybrids are only about 3 cm. taller than the tall parents. There is thus no hybrid vigour with respect to this character.

The increase in branching of hybrids, in Jones's material, over the higher tillering parent is considerable showing unmistakable evidence of heterosis, the hybrids producing from 2 to 5·8 tillers more. In our case only one cross showed increase in tillering which was insignificant. There is no evidence of heterosis in tillering in our material.

Our results agree with Jones as regards length of panicles for which no hybrid vigour was observed.

Jones observed considerable vigour in yield of hybrids. In our case heterosis was observed in two cases out of five. In one case an increase of 39·41 per cent (for $n = 7$) was not significant. In this case the yield of the F_1 plants was very variable (variance = 15·176) ; and this may account for the non-significance of the average yield of F_1s over the more prolific parent.

The weight of the whole plant did not show any hybrid vigour.

SUMMARY

1. Hybrid vigour was not observed with respect to height of plants, tillering, panicle length and weight of the whole plant.

2. In some crosses hybrid vigour in yield was observed.

REFERENCES

Fisher, R. A. (1932). 'Statistical Methods for Research Workers', Oliver & Boyd, London.

Jones, J. W. (1926). *Jour. Amer. Soc. Agron.* **18**, 423-428.

INHERITANCE OF INNER GLUME COLOUR IN RICE

BY
S. K. MITRA, I.A.S.
Economic Botanist, Assam
AND
P. M. GANGULI
Botanical Assistant

(Received for publication on 21st July 1936)

(With Plates V and VI)

In a previous publication [Mitra, Gupta, Ganguli, 1928] the writers dealt with the various simple-colour factors that played an important part in the distribution of colour in the main parts of the rice plants both in regard to vegetative and floral characters. The present paper is a continuation of the same work in detail at the Rice Station at Karimganj, Assam, dealing with the inner glume colour alone.

Some of the prominent inner glume colours in rice in the floral stage are green, brown, and blackish green with green ribs and base which ripen yellow, deep brown and blackish brown, respectively. But in some cases these green and blackish green colours turn black just before maturity which can be noticed with careful observation. Several crosses were tried to study the inheritance of these characters, the results of which are described below.

I. Green × Brown (Crosses Nos. 811 and 869)

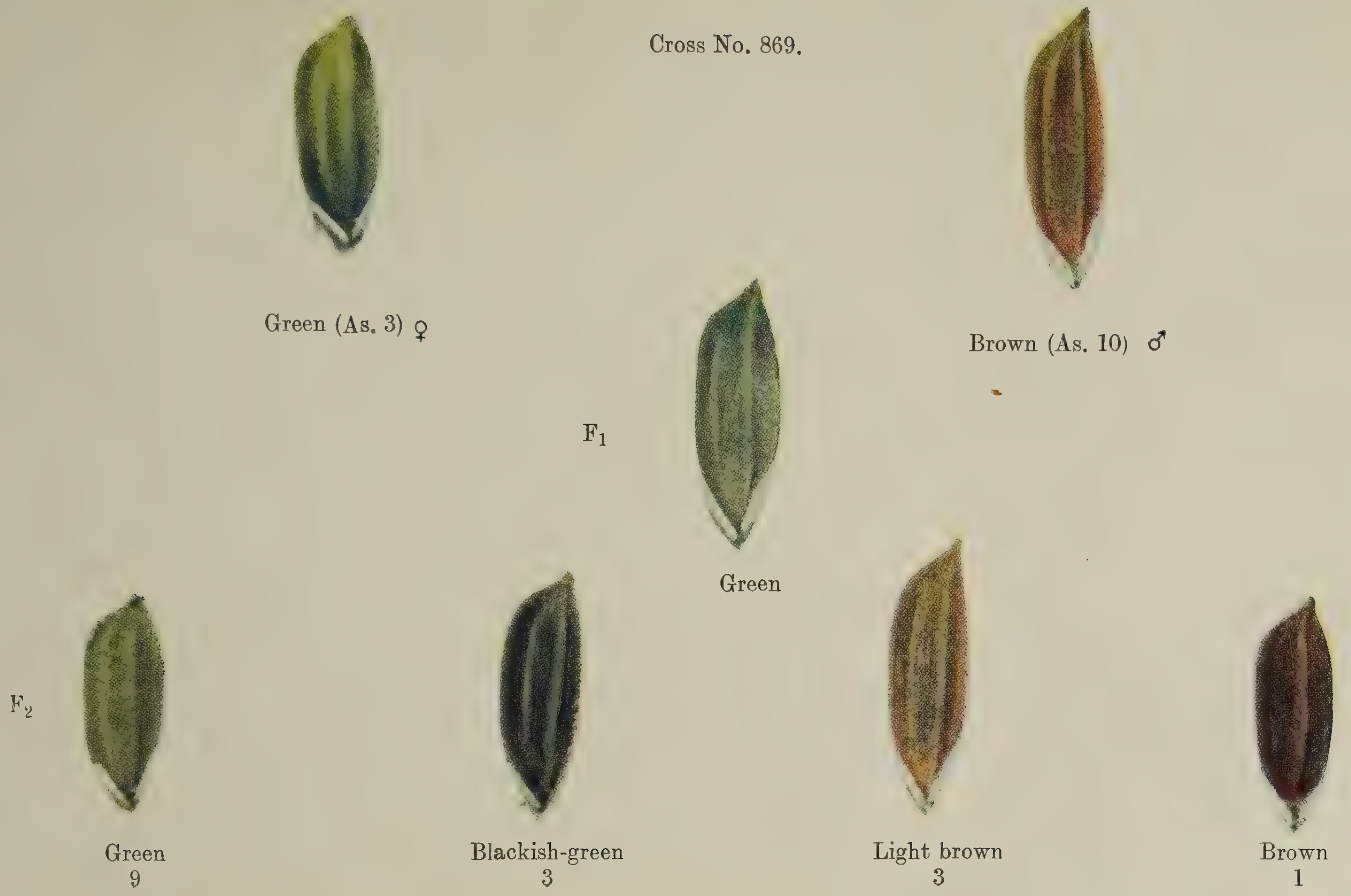
Two crosses were tried between As 2, As 3, and As 10. Of these As 2 *Kasalath* and As 3 *Basmati* were green and As 10 *Kataktara* was brown in the floral stage. In each case the F_1 was green and in F_2, apart from the two parental characters (green and brown), two new characters, blackish green and light brown, were also obtained in the ratio of 9 green : 3 blackish green : 3 light brown : 1 brown (Plate V) as shown in Table I.

Table I

Cross No.	Parentage	Phenotypes	Green	Blackish green	Light brown	Brown	Total	χ^2	P
811	As 2 × As 10	Observed . .	141	42	45	13	241	0·726	0·87
		Calculated 9 : 3 : 3 : 1	135·56	45·19	45·19	15·06	241		
869	As 3 × As 10	Observed . .	126	60	42	18	246	0·089	0·11
		Calculated 9 : 3 : 3 : 1	138·6	46·2	46·2	15·4	246		

PLATE V

Cross No. 869.


Green (As. 3) ♀
Brown (As. 10) ♂
F_1
Green
F_2
Green
9
Blackish-green
3
Light brown
3
Brown
1

The fit is very good in cross No. 811, while in cross No. 869 although it was not quite good the ratio was confirmed in F_3 as will be shown later. The appearance of blackish green and light brown characters may be explained by assuming two factors **C** and **L**, representing the blackish green and light brown colours respectively. It may now be supposed that **C** and **L** together will make the colour green and their total absence will make it brown.

In the light of this assumption the following genetic constitution of the parents and the F_1 may be formulated.

Parents	As 2 or As 3	**CCLL**	Green.
	As 10	**ccll**	Brown.
	F_1	**CcLl**	Green.

The factorial analysis of the F_2 segregations and their expected behaviour in F_3 are shown below:

F_2		F_3 behaviour
Phenotypes	Genotypes	
9 **CL** green	1 **CCLL**	pure green.
	2 **CCLl**	3 green : 1 blackish green.
	2 **CcLL**	3 green : 1 light brown.
	4 **CcLl**	9 green : 3 blackish green : 3 light brown : 1 brown.
3 **Cl** blackish green	1 **CCll**	pure blackish green.
	2 **Ccll**	3 blackish green : 1 brown.
3 **cL** light brown	1 **ccLL**	pure light brown.
	2 **ccLl**	3 light brown : 1 brown.
1 **cl** brown	1 **ccll**	pure brown.

All the above segregations have been obtained in F_3 in both the crosses which only confirm the F_2 ratio. Tables II and III will show the data of F_3 results clearly in details.

It may also be mentioned that in the mature stage the green turned yellow and the blackish green turned blackish brown, while the rest were as above.

Parnell *et al.* [1917] working with the inner glume colour of rice obtained several ratios, such as, 3 green : 1 gold, 1 green : 2 patchy gold : 1 gold, 3 green : 1 dark furrows, and others. The brown, light brown and blackish green glumes of the authors are perhaps the same as what has been described by Parnell *et al.* [1917, 1922] as gold, ripening gold and dark furrow types respectively. Although the ratios obtained above are not the same as that in our case an interpretation may be made out to bring them up to the same category.

TABLE II

Cross No. 811

Nature of F$_2$ parents	F$_3$ behaviour	Number of cultures		F$_3$ frequencies				Total number of plants	χ^2	P
		Expected	Observed	Green	Blackish green	Light brown	Brown			
42 green . .	Pure green . .	4·67	4	Pure	...	...	...	...	...	...
	3 green : 1 blackish green . . .	9·34	9	342	97	...	...	439	1·97	0·17
	3 green : 1 light brown	9·34	11	409	...	120	...	529	1·52	0·22
	9 green : 3 blackish green : 3 light brown : 1 brown . .	18·68	18	532	151	148	50	881	6·17	0·11
12 blackish green	Pure blackish green .	4·0	2	...	Pure	...	...	...	...	...
	3 blackish green : 1 brown . . .	8·0	10	...	372	...	114	486	0·61	0·45
14 light brown .	Pure light brown .	4·67	3	...	...	Pure	...	...	...	...
	3 light brown : 1 brown	9·34	11	...	...	405	130	535	0·14	0·71
6 brown . .	Pure brown . .	6	6	...	...	...	Pure	...	...	...

TABLE III

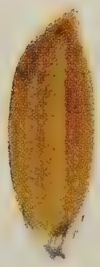
Cross No. 869

Nature of F$_2$ parents	F$_3$ behaviour	Number of cultures		F$_3$ frequencies				Total number of plants	χ^2	P
		Expected	Observed	Green	Blackish green	Light brown	Brown			
42 green . . .	Pure green . .	4·67	4	Pure	...	...	...	...	...	...
	3 green : 1 blackish green . . .	9·34	9	338	102	...	...	440	0·776	0·4
	3 green : 1 light brown	9·34	8	304	...	86	...	390	1·808	0·18
	9 green ; 3 blackish green : 3 light brown : 1 brown . .	18·68	21	591	199	175	61	1,026	2·284	0·52
23 blackish green	Pure blackish green .	7·67	10	...	Pure	...	...	...	...	...
	3 blackish green : 1 brown . . .	15·34	13	...	482	...	154	636	0·209	0·66
14 light brown .	Pure light brown .	4·67	3	...	...	Pure	...	...	...	...
	3 light brown : 1 brown	9·34	11	...	...	401	134	535	0·0006	0·98
10 brown . .	Pure brown . .	10	10	...	...	...	Pure	...	...	...

II. GREEN × BLACKISH GREEN (CROSS NO. 777)

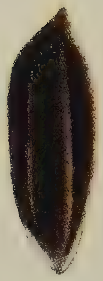
Only one cross was tried with the above characters between As 3 *Basmati* and D 138/6 *Tepi Dumai*. In the floral stage the glumes of As 3 were green and those of D 138/6 were blackish green with green ribs and base. As 3 ripened

Cross No. 777.
(mature)



♀

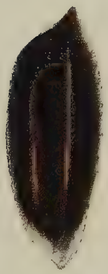
Yellow (As. 3)



♂

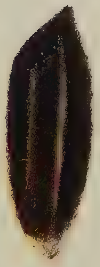
Black (D. $\frac{138}{6}$)

F$_1$

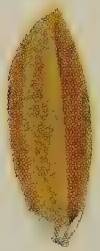


Black

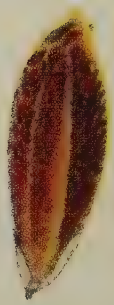
F$_2$



Black
36



Yellow
21



Blackish-brown
7

yellow and D 138/6 ripened black. In the floral stage the F_1 was green and in F_2 the segregation was in the ratio of 3 green : 1 blackish green as shown below :—

		Green	Blackish green	Total
Cross No. 777 (Floral stage)	Observed	209	55	264
	Calculated (3 : 1)	198	66	264

$\chi^2 = 2\cdot444. \quad P = 0\cdot12$

Although the fit is not quite good, the ratio has, however, been confirmed in F_3. Out of the 264 plants only 110 plants were grown in F_3 and their behaviour is shown below in Table IV.

Table IV

Cross No. 777 (As 3 × D 138/6)

Nature of F_2 parents	F_3 behaviour		
	Segregation	Observed	Expected
92 green	Pure green	33	30.67
	Segregating (3 : 1)	59	61.34
18 blackish green	Pure	18	18

In the maturing stage, however, there was an entirely different ratio. The green F_1 glumes ripened black. Some of the greens and blackish greens in F_2 ripened yellow and blackish brown respectively, while the majority ripened black, giving a tri-hybrid ratio of 36 black: 21 yellow: 7 blackish brown (Plate VI) as shown below :

		Black	Yellow	Blackish brown	Total
Cross No. 777 (Mature stage).	Observed	159	82	23	264
	Calculated (36 : 21 : 7)	148.5	86.59	28.86	264

$\chi^2 = 2\cdot168. \quad P=0.35.$ The fit is good.

The tri-hybrid ratio here can be explained on the basis of two factors, $\mathbf{B}_1$ and $\mathbf{B}_2$ for black and one factor, **Y**, for yellow colour. The factors $\mathbf{B}_1$ and $\mathbf{B}_2$ together are responsible for the black colour, and **Y** alone or in combination with either $\mathbf{B}_1$ or $\mathbf{B}_2$ will bring out the yellow. In the absence of **Y** the colour will be blackish brown. $\mathbf{B}_1$ and $\mathbf{B}_2$ are together dominant over **Y**, and **Y** is dominant over either

B_1 or B_2. The genetic constitution of the parent plants and the F_1 may be formulated as shown below :—

Parents	D 138/6	$B_1B_1B_2B_2yy$	Black.
	As 3	$b_1b_1b_2b_2YY$	Yellow.
	F_1	$B_1b_1B_2b_2Yy$	Black.

The factorial analysis of the F_2 segregation with their expected behaviour in F_3 is shown below :—

	F_2		F_3 behaviour
	Phenotypes	Genotypes	
36 black	27 B_1B_2Y black	1 $B_1B_1B_2B_2YY$	pure black.
		2 $B_1b_1B_2B_2YY$	3 black : 1 yellow.
		2 $B_1B_1B_2b_2YY$	3 black : 1 yellow.
		2 $B_1B_1B_2B_2Yy$	all black.
		4 $B_1b_1B_2b_2YY$	9 black : 7 yellow.
		4 $B_1b_1B_2B_2Yy$	12 black : 3 yellow : 1 blackish brown.
		4 $B_1B_1B_2b_2Yy$	12 black : 3 yellow : 1 blackish brown.
		8 $B_1b_1B_2b_2Yy$	36 black : 21 yellow : 7 blackish brown.
	9 B_1B_2y black	1 $B_1B_1B_2B_2yy$	pure black.
		2 $B_1B_1B_2b_2yy$	3 black : 1 blackish brown.
		2 $B_1b_1B_2B_2yy$	3 black : 1 blackish brown.
		4 $B_1b_1B_2b_2yy$	9 black : 7 blackish brown.
21 yellow	9 B_1b_2Y yellow	1 $B_1B_1b_2b_2YY$	pure yellow.
		2 $B_1B_1b_2b_2Yy$	3 yellow : 1 blackish brown.
		2 $B_1b_1b_2b_2YY$	all yellow.
		4 $B_1b_1b_2b_2Yy$	12 yellow : 4 blackish brown.
	9 b_1B_2Y yellow	1 $b_1b_1B_2B_2YY$	pure yellow.
		2 $b_1b_1B_2b_2Yy$	all yellow.
		2 $b_1b_1B_2B_2Yy$	3 yellow : 1 blackish brown.
		4 $b_1b_1B_2b_2Yy$	12 yellow : 4 blackish brown.
	3 b_1b_2Y yellow	1 $b_1b_1b_2b_2YY$	pure yellow.
		2 $b_1b_1b_2b_2Yy$	3 yellow : 1 blackish brown.
7 blackish-brown	3 b_1B_2y blackish-brown	1 $b_1b_1B_2B_2yy$	pure blackish brown.
	3 B_1b_2y blackish brown	2 $b_1b_1B_2b_2yy$	all blackish brown.
		1 $B_1B_1b_2b_2yy$	pure blackish brown.
	1 b_1b_2y blackish brown	2 $B_1b_1b_2b_2yy$	all blackish-brown.
		1 $b_1b_1b_2b_2yy$	pure blackish brown.

All the above segregations are obtained in F_3 as shown in detail in Table V.

TABLE V

Cross No. 777

Nature of F_2 parents	F_3 behaviour	Number of cultures		F_3 frequencies			Total number of plants	χ^2	P
		Expected	Observed	Black	Yellow	Blackish brown			
65 black	Pure black	7·22	9	Pure	...	...	...	...	...
	3 black : 1 yellow	7·22	12	435	152	...	587	0·251	0·63
	9 black : 7 yellow	7·22	11	318	220	...	538	1·792	0·19
	12 black : 3 yellow : 1 blackish brown	14·44	11	401	98	40	539	1·294	0·53
	36 black : 21 yellow : 7 blackish brown	14·44	13	375	190	73	638	2·666	0·27
	3 black : 1 blackish brown	7·22	5	174	...	71	245	2·069	0·16
	9 black : 7 blackish brown	7·22	4	111	...	84	195	0·035	0·86
38 yellow	Pure yellow	12·0	13	...	Pure	...	...	...	...
	3 yellow : 1 blackish brown	24·0	25	...	907	317	1,224	0·527	0·48
7 blackish brown	Pure blackish brown	7	7	...	...	Pure	...	...	...

The ripening black colour of the mature inner glume is rather independent of the colours in the flowering stage and develops on either green or blackish green colour but not on the brown. Moreover, the ripening black colour is dominant over either yellow or blackish green in the mature stage. Hence the two sets of factors have been treated separately.

As in both the above crosses (Cross Nos. 869 and 777) the As 3 *Basmati* was common the relation between the factors determining the colour of the inner glume at the flowering stage and those determining at the mature stage may well be stated here. In the floral stage of the first cross (Cross No. 869) the F_1 greens segregated into 9 green : 3 blackish green : 3 light brown : 1 brown, while in the mature stage the greens changed to yellows, and blackish greens to blackish browns and the rest without any change as has been mentioned. So, there was no change in the ratio from the flowering to the mature stage. In the second cross (Cross No. 777), on the other hand, the F_1 greens in the floral stage segregated in a simple ratio of 3 green : 1 blackish green as already shown. But in the mature stage, on account of the ripening black character of the other parent, D 138/6, a tri-hybrid ratio entered which changed the genetic constitution in F_2. Consequently, a divergent assortment of combinations was obtained where the simple 3 : 1 ratio was broken up into a complex triplicate ratio in which the dominant greens were transformed to both black and yellow and the blackish greens to both black and blackish brown segregating in the ratio of 9 : 7 in each case, thus finally bringing out a ratio of 36 black : 21 yellow : 7 blackish brown as shown below :—

	Parents D 138/6 × As 3		F_1	F_2
Floral stage .	(Blackish green)	(Green) .	Green .	Green 3 (48) : Blackish green 1 (16)
Mature stage	(Black)	(Yellow)	Black	27 : 21 9 : 7 Black Yellow Blackish brown $\underbrace{27+9}_{36}$: 21 : 7

The following table will actually show the green segregating into 9 black : 7 yellow and blackish green into 9 black : 7 blackish brown in the mature stage :—

TABLE VI

Cross No. 777

Transformation of inner glume colour from the flowering to the mature stage in F_2

Flowering stage	Mature stage	Observed frequencies			Total number of plants	χ^2	P
		Black	Yellow	Blackish brown			
209 green . . .	9 black : 7 yellow . . .	127	82	...	209	1·718	0·2
55 blackish brown .	9 black : 7 blackish brown .	31	...	24	55	0·074	0·8

Rangaswami and others [1934] also obtained a similar ratio in the grain colour of sorghum with 36 brown : 21 red : 7 yellow.

Parnell *et al.* [1917, 1922] obtained both 3 : 1 and 9 : 7 ratios for ripening black to ripening straw and 3 : 1 for straw to black furrows.

III. BLACK × BLACKISH BROWN (CROSS NO. 1036)

In another cross between the above two grain characters the types D 138/6 *Tepi Dumai* and D 36/14 *Baurash Dumai* were used. Both the types were blackish green with green ribs and base in the floral stage and could not be ordinarily distinguished. In the mature stage, however, D 138/6 ripened black and D 36/14 ripened blackish brown. The F_1 was black and in F_2 the segregation was in the ratio of 3 black : 1 blackish brown as shown below :—

		Black	Blackish brown	Total
Cross No. 1036 .	Observed . . .	251	87	338
	Calculated (3 : 1) .	253·5	84·5	338

$\chi^2 = 0{\cdot}098$. $P = 0{\cdot}76$

The fit is very good. The genetic constitution of the parent plants may, therefore, be supposed to be as shown below :—

D 138/6	$B_1B_1B_2B_2yy$	Black.
D 36/14	$B_1B_1b_2b_2yy$ or $b_1b_1B_2B_2yy$	Blackish brown.

This is rather in confirmation of the previous cross. Out of the 338 F_2 plants only 95 were grown in F_3 which confirmed the F_2 ratio as shown in the table below:-

TABLE VII

Nature of F_2 parents	F_3 behaviour		
	Segregations	Observed	Expected
72 black	Pure	25	24
	Segregating (3 : 1)	47	48
23 blackish brown	Pure	23	23

SUMMARY

The factors determining the inheritance of inner glume colour in rice have been studied in three crosses. The factors **C** and **L** are responsible for the production of blackish green and light brown colours, respectively. The presence of both will make the colour green and their total absence will make it brown in the ratio of 9 green : 3 blackish green : 3 light brown : 1 brown.

The black colour of the mature inner glume is the result of the combined effects of the factors $\mathbf{B}_1$ and $\mathbf{B}_2$, while **Y** alone or in combination with either $\mathbf{B}_1$ or **B** will make the colour yellow. The blackish brown colour will appear in the absence of **Y**. $\mathbf{B}_1$ and $\mathbf{B}_2$ are together dominant over **Y**, and **Y** is dominant over either $\mathbf{B}_1$ or $\mathbf{B}_2$. The F_2 segregation is in the ratio of 36 black : 21 yellow : 7 blackish brown. The F_3 behaviour has also conformed to the expected segregations.

REFERENCES

Mitra, S. K., Gupta, S. N. and Ganguli, P. M. (1928). *Mem. Dept. Agric. Ind.* (*Bot. Ser.*) **15**, No. 4.

Parnell, F. R., Rangaswami Ayyangar, G. N., and Ramiah, K. (1917). *Mem. Dept. Agric. Ind.* (*Bot. Ser.*) **9**, No. 2.

———————— (1922). *Mem. Dept. Agric. Ind.* (*Bot. Ser.*) **11**, No. 8.

Rangaswami Ayyangar, G. N., Vijiaraghavan, C., Sankara Ayyar, M.A., Panduranga Rao, V., and Subramanyam, P. (1934). *Ind. Jour. Agric. Sci.* **4**, Pt. I.

INHERITANCE OF SHEATHED EAR IN RICE

BY

R. L. SETHI, M.Sc., B.Sc. (Edin.), I.A.S.

Economic Botanist (Sugarcane and Paddy) to Government, United Provinces

B. L. SETHI, M.Sc., Ph.D. (Wales)

Assistant Paddy Specialist

AND

T. R. MEHTA, B.Sc.

Research Assistant, Rice Research Station, Nagina

(Received for publication on 20th August 1936)

(With Plates VII—IX)

Introduction

This paper deals with the inheritance of a peculiarity in rice consisting in the retention of the panicle within the leaf-sheath. This peculiarity occurs in one of the varieties known as *Sathi*, grown extensively in the United Provinces. The crops of *Sathi* escape the attacks of the rice fly, *Leptocorisa varicornis*, which would otherwise suck the developing rice grains, as it does in the case of all the other varieties where the grains are not protected. The inheritance of this protective device, which will henceforth be termed as 'sheathed ear', was studied in some of the crosses between *Sathi* and the 'open' types.

The problem

The most urgent and baffling problem that faces the rice growers in the United Provinces is that of saving the crop (especially the early crop) from the ravages of the rice fly, *Leptocorisa varicornis*, locally known as *gundhi*. It is a green insect emitting an evil odour which has given to it its local name (*gundh* = stink). In certain epidemic years it causes extensive damage. It is most active from the middle of August to the middle of October, and vanishes in the beginning of the cold weather, in November. No method of control has so far been devised. The crude method of 'catch and kill' has been only meagrely successful, because it is both a slow as well as a comparatively ineffective method. The only rice which escapes the attack of *gundhi* is *Sathi*, which is a group of coarse-grained, poor-yielding, early-ripening, broadcast rices. In all the other varieties the ears are exposed, with the result that during grain formation these varieties offer a rich and tempting feast to *gundhi*. Such being the case the only wise course left to the cultivator to enable him to save his varieties is either to grow late-maturing types which fruit when *gundhi* has disappeared, or to grow the poor *Sathi*

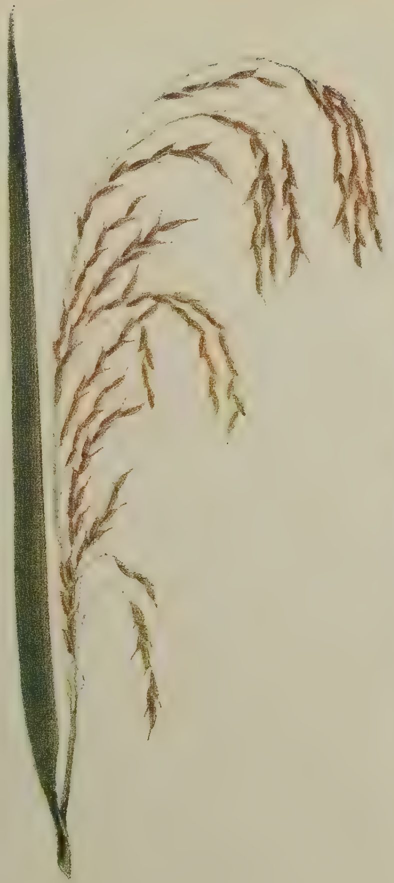


FIG. 1. A healthy ear of the 'open' fine-grained parent (T. 1) as it looks if the insect *gundhi* (*Leptocorisa varicornis*) does not attack it.

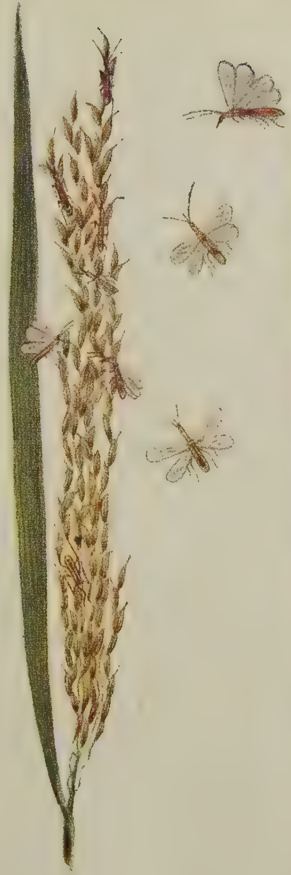


FIG. 2. An immature ear of T. 1 being attacked by the insects.

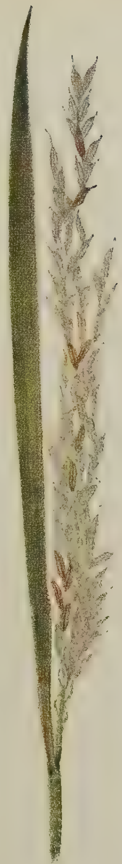


FIG. 3. The result of the attack. Most of the grains are left behind as empty husks.

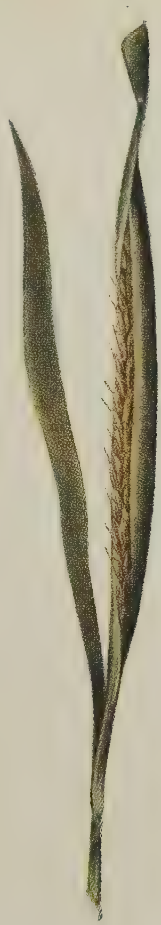


FIG. 4. An ear of one of the extracted fine-grained hybrids, immune to the attack of the insect because of the sheathed ear character.

types. He chooses the alternative which suits him, while the rich and varied collection of the fine, early rices (including the famous *Bansmati* varieties of Dehra Dun) has to be left untouched, except by a few enterprising people who cater for the rich tastes of the wealthier and more fashionable classes. By charging high prices for these fine rices they manage to run their business at profit, but the unsophisticated and poor cultivator would fight shy of such an enterprise. It would not be in his line, and would, besides, be beyond his means.

The problem, then, was to effect a general improvement of the rice crop of the provinces by encouraging the cultivation of the finer early-ripening rices. This became still more important with the advent of the canals when the demand for heavy-yielding early-ripening crops increased greatly for rotation with the *Rabi* crops. The only method that appeared feasible was that of conferring immunity to rice fly upon these rices through hybridization with *Sathi*. It was hoped that some of the resulting hybrids would combine in them the protective sheath of *Sathi* with the good features (yield, fine grain, etc.) of the finer rices, and thus receive preference over *Sathi* rices which are at present widely grown in spite of their coarse grain and poor yield.

The amount of damage caused by insect and the way it is hoped to check its attack are illustrated in Plate VII, which shows ears of the susceptible parent and an ear of one of the immune fine-grained hybrids.

CROSSES MADE

The problem was taken up initially by the Economic Botanist at Cawnpore in 1929 and a few crosses were made between a *Sathi* type (T 131) and a few of the fine-grained types (T 1, T 3, T 7 and T 12). The contrast in the characters of T 131 and of the other types was remarkable. For example, T 131 is very short, leaf-sheath lightly coloured purple, apiculus and stigma purple, grain short and coarse with light red kernel and dark grey husk on ripening. The ears are always enclosed in the sheath and the grain sheds easily if the sheath is removed. Types 1, 3, 7 and 12 are tall compared to T 131 and the vegetative and floral organs are without colour. The grain is long and fine, with white kernel and straw-coloured husk (except in T 1 which has orange-coloured husk). The grain does not shed unless the ears are threshed. Plate IX roughly gives an idea of the differences existing in the parents.

The F_1 was grown in 1930 and the F_2 in 1931 at Cawnpore after which the work was transferred to the Rice Research Station, Nagina, where the F_3 and later generations were grown. Two crosses, *viz.*, T 1 × T 131 and T 7 × T 131 were made again in 1931 and their F_2 and F_3 generations grown in 1933 and 1934, respectively. The hybrids of the crosses made in 1929 have now reached the F_7 stage, and most of them possess the protective ear-sheath.

Incidentally it may be mentioned here that the study of the different characters has revealed the fact that many of them (*e.g.*, plant height, earliness, grain size, etc.) followed a quantitative mode of inheritance. This, coupled with the fact that the parents were so contrasted in their characters, has resulted in continued segregation of characters year after year. Consequently very few of them have been absolutely 'fixed', and continued selection alone will make it possible for us to purify more of them. Of a few of the fixed hybrids two have been selected for yield tests in replicated beds.

In this paper, however, we shall confine ourselves to a record of the inheritance studies on the 'sheathed ear' character, which were pursued not only because of the practical importance of the studies but also because no literature on the subject seems to exist. The data presented here may, therefore, prove of interest to future workers on this character.

PARENTS AND F_1

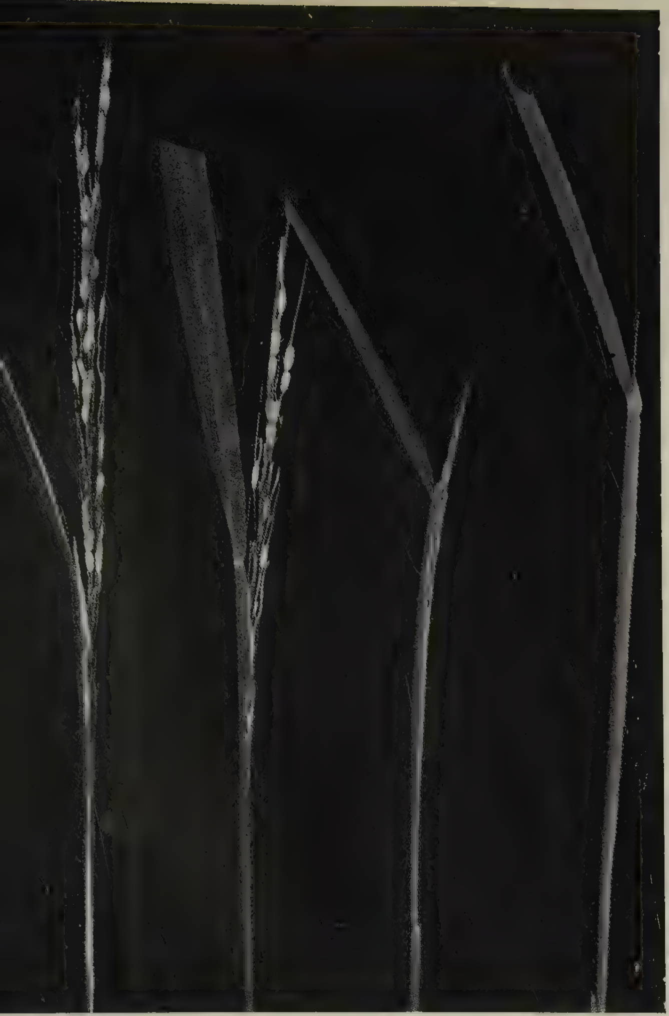
The two parents used in each cross represent two extreme expressions of the sheath character, one parent (T 131) having the ears completely enclosed and the other (T 1 or the other types) having the ears completely emerged. Under broadcast conditions the ears never emerge in T 131. Under transplanted conditions usually one or two tillers in each plant bear ears the tips of which emerge from the sheath. Among the hybrids the phenotypes possessing a sheath like that of T 131 have been termed 'enclosed'. The completely open-eared phenotypes like T 1, T 3, T 7 and T 12 have been termed 'emerged'. The two parents of a cross (T 131 × T 7) and the F_1 are shown in Plate IX. The F_1 was very vigorous and as tall as or even slightly taller than the taller parent. The vegetative and the floral parts were coloured as in T 131. The grain was coarse and dark like that of T 131 and shed easily. The ears were, however, emerged as in the other parents, showing that the 'emerged' condition was dominant. Plate VIII, fig. 2 shows the parental and F_1 ears of another cross. Of the two ears of T 131 shown in this figure one (*viz. a*) has been shown in the normal condition (*i.e.*, enclosed) while the other (*viz. b*) has been exposed by tearing the sheath apart.

The F_1, it will be seen in Plate VIII, fig. 2, is 'emerged'. However, it was different from the 'emerged' parent in certain respects. The exsertion of the ear was not so pronounced as that of the parent, while many of the ears, especially those on the later-formed tillers, were only partially emerged. In fact, the variation in the sheath character was striking, some of the ears being completely enclosed. Plate VIII, fig. 1 shows F_1 ears partially or completely hidden in the sheath. Usually about fifty per cent of the ears were emerged while the rest were only partially emerged or were enclosed.

F_2 GENERATION

The four F_2 generations grown in 1931 at Cawnpore belong to different crosses, *viz.*, (1) T 131 × T 1; (2) T 131 × T 7; (3) T 131 × T 7 and (4) T 131 × T 12.

PLATE VIII



G. 1. Later-formed ears of an F_1 plant showing different degrees of enclosure of ears in the sheath.

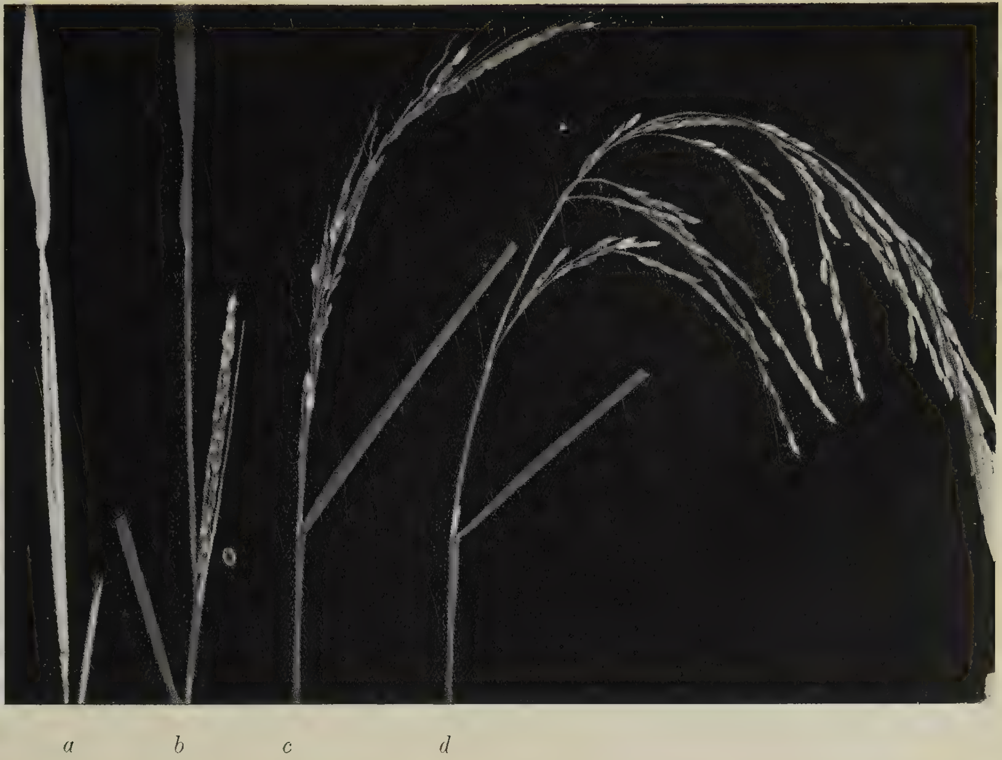


FIG. 2. Ears of T. 131 (*a* and *b*), T. 3 (*d*) and F_1 (*c*).

The populations in the four crosses were, respectively, 175, 248, 696 and 627. Another F_2 population of 701 plants was studied at Nagina in 1933.

In this generation were obtained plants of vastly different kinds, the majority representing the parents and the F_1 in sheathed ear. Before describing the various phenotypes into which the F_2 population was divided it may be emphasized that the character varied a great deal on the same plant in certain hybrids. Without stopping to discuss why individual hybrids showed such variations it may be stated that roughly the following phenotypes could be recognised.

(*1*) *Emerged.*—All or most of the ears completely emerged ; a few ears partly or half enclosed. In the late-formed tillers an occasional enclosed ear also present.

(*2*) *Partially emerged.*—Most of the ears half enclosed, a few ears showing only the tips, a completely emerged or enclosed ear not uncommon.

(*3*) *Tips emerged.*—Most of the ears showing only the tips ; here and there an ear half emerged or completely enclosed also present.

(*4*) *Enclosed.*—The ears completely enclosed. The earliest-formed tiller may bear a slightly emerged ear.

Class (1) corresponds to the finer, ' open ' parents and the F_1, while class (4) corresponds to the *Sathi* parent. Classes (2) and (3) represent new phenotypes. Cases which could not be placed with confidence in one or the other class were common.

In studying the sheathed ear character it was expedient to make observations upon the plants when the character had assumed its final expression. It is apparent that observation made too early would give a different picture of the plant in cases where the earlier-formed tillers differed from the later-formed tillers in this character.

Although the ' emerged ' and the ' partially emerged ' classes were kept separate when the observations were being made it was found that there were many intermediate forms which could with equal certainty be placed in one or the other class. It was considered desirable to combine the two classes. The F_2 population of cross T 1 × T 131 studied in 1933 gave the following frequencies :—

	' Emerged ' and ' Partially emerged '	' Tips emerged '	' Enclosed '
Observed frequency	485	59	157
Calculated frequency (on 12 : 1 : 3 basis)	525·75	43·81	131·44

The frequencies suggested a dihybrid mode of inheritance, the three groups being in the ratio 12 : 1 : 3. The observed values have been tested by the ' goodness-of-fit ' test.

Goodness-of-fit test

	' Emerged ' and ' Partially emerged '	' Tip emerged '	' Enclosed '
$o - c$	40.75	15.19	25.56
$(o - c)^2$	1660.56	230.74	653.31
$\frac{(o - c)^2}{c}$	3.16	5.27	4.97

$\chi^2 = 13.40$

(o = observed frequency and c = calculated frequency).

Fisher's table of χ^2, which is a very convenient modification of Elderton's well-known χ^2 table [Fisher, 1932], gives values of χ^2 corresponding to specially selected values of P (*i.e.*, probability). The value of P when $n = 2$ and when $\chi^2 = 13 \cdot 40$ is below $0 \cdot 01$, meaning thereby that the discrepacny between the observed and calculated values is large and is open to suspicion. The difficulty of accurately classifying the F_2 population should not be forgotten in this connection. This may have disturbed the fit, as is corroborated by observations made in the F_3 generation.

The diverse types of ears obtained in the F_2 are illustrated in Plate IX, fig. 2, which represents the parental, F_1 and F_2 generations of cross T 131 × T 7. The F_1 has been represented by two ears, one ' emerged ' and the other ' tip emerged ', the latter being produced on one of the later-formed tillers. The F_1 ears are without spikelets, the grain-shedding habit of T 131 being dominant in inheritance. The F_2 is represented by ears ranging from the ' emerged ' to the ' enclosed ' condition. In every plant the ears had to be bagged in order to collect the shed grains for purposes of yield-study and of raising the F_3 generation.

F_3 GENERATION

The F_3 generation was closely studied in cross T 131 × T 1 in 1934. The number of cultures examined was forty-two, the size of population varying from fifty to a hundred plants in the different cases. The segregations observed were varied, and were something like the following. Some of the cultures consisted only of the ' open ' plants, but the emergence of the ears was not quite like that of the ' open ' parent, because while many plants bore both completely and incompletely emerged ears, some plants were inclined towards the ' partially emerged ' class. Some cultures contained only ' emerged ' and ' partially emerged ' plants though classifiable as such with difficulty. Certain other cultures showed segregation like that of the F_2. In certain others there was a preponderance of ' enclosed ' or ' tip emerged ' plants over ' emerged ' plants, segregations which would indicate a reversal of dominance of the ' emerged ' habit. In some cases the majority of plants belonged to the ' partially emerged ' class only a small minority

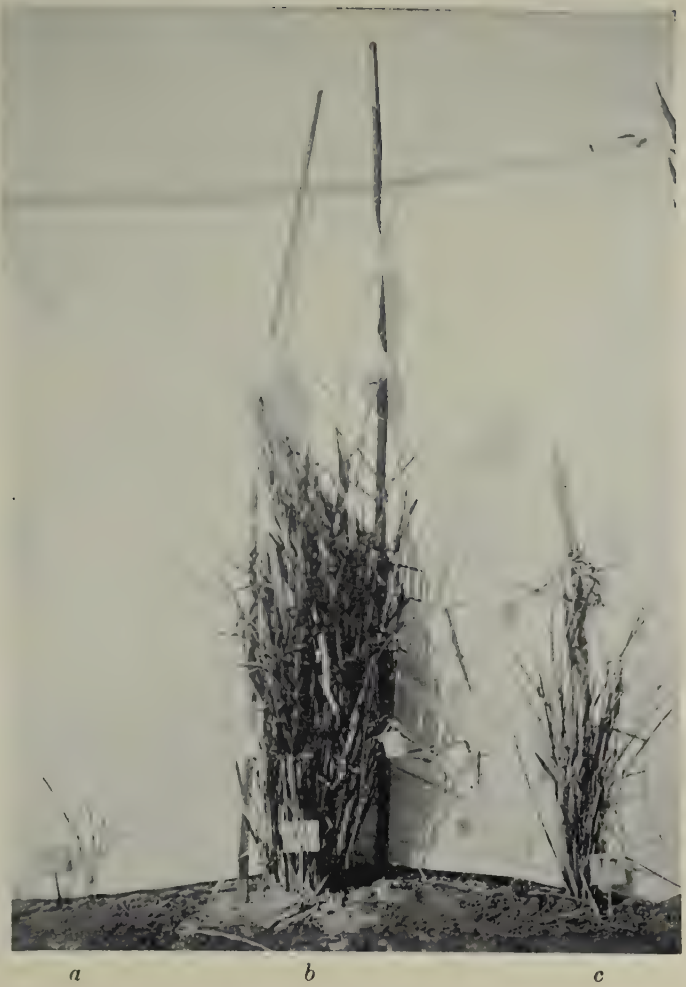


FIG. 1. Parents (*a* and *c*) and F_1 (*b*) of cross T131 × T7.

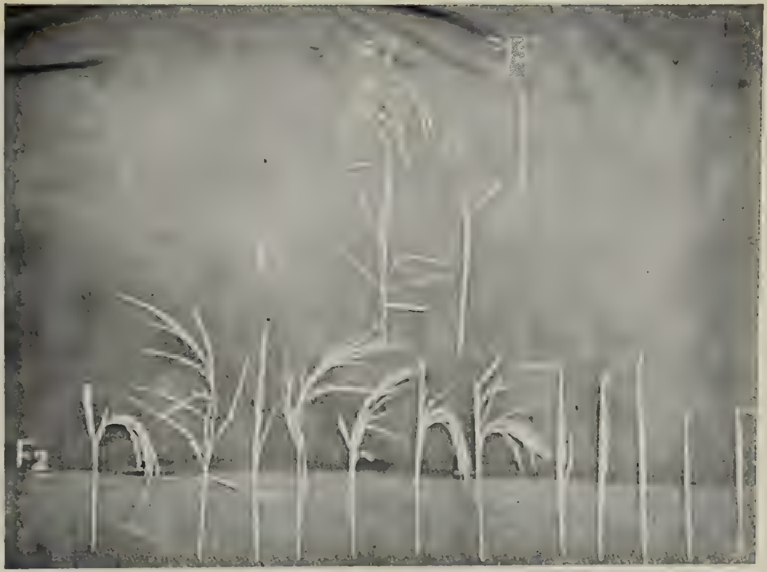


FIG. 2. Sheathed ear in the parental, F_1 and F_2 generations of cross T131 × T7.

of plants being ‘ emerged ’ or ‘ enclosed ’. Cultures where the plants were ‘ emerged ’ and ‘ enclosed ’ while the intermediate phenotypes were altogether absent were also encountered. Cultures pure for the different conditions were also present. The data have been presented in detail in the Appendix.

The diversity of segregation observed appeared baffling. The dominance of ‘emerged ’ habit in one case and its recessiveness in another appeared contradictory. The failure of the ‘ tip emerged ’ class—the supposed double recessive phenotype—to breed pure was perplexing. A careful review of the data helped to dispel much of the confusion and contradiction. One fact emerged, that the expression of the sheathed ear character in F_2 was not entirely dependent upon the genes, but was controlled to some extent by the environment. That this was so was evidenced by the fact that some of the F_2 plants classified as ‘ enclosed ’ were really ‘ partially emerged ’ as shown by their breeding behaviour in F_3. Similarly some of the ‘ tip emerged ’ plants should rightly have been classified as ‘ enclosed ’. The F_2 frequencies corrected on the basis of the F_3 observations will be presented later. Another fact which became clear was that the inheritance of the sheath habit was trihybrid and not dihybrid as was indicated in the F_2. Considered on a trihybrid basis the data of the two generations fall in line with the theory advanced. We may first present the theory. The discussion of the data can then be considered in its proper bearing.

THEORY

We may suppose that the basic condition in paddy is the ‘ open ’ or the ‘ emerged ’ condition. A gene **P** causes the ‘ partially emerged ’ condition and a gene **T** causes the ‘ tip emerged ’ condition. **P** and **T** together cause complete emergence. There is a third gene **E** which causes the ‘ enclosed ’ condition. It is completely dominant over **T**, only partially so over **P**, but not over **PT** (*i.e.*, when **P** and **T** come together). Thus the ‘ emerged ’ class can be represented by the formulæ **PTe**, **PTE** or **pte**, the ‘ partially emerged ’ class by **Pte** or **PtE**, the ‘ tip emerged ’ class by **pTe** and the ‘ enclosed ’ class by **pTE** or **ptE**.

The facts of the cross may now be represented by the following scheme.

Parents :		**PPTTee** T 1	× **ppttEE** T 131					
F_1 :		**PpTtEe**						
F_2 :	1 **PPTTEE**	1 **PPTTee**	1 **PPttEE**	1 **ppTTEE**	1 **PPttee**	1 **ppTTee**	1 **ppttEE**	1 **ppttee**
	2 **PPTTEe**	2 **PPTtee**	2 **PPttEe**	2 **ppTTEe**	2 **Ppttee**	2 **ppTtee**	2 **ppttEe**	
	2 **PPTtEE**	2 **PpTTee**	2 **PpttEE**	2 **ppTtEE**				
	2 **PpTTEE**	4 **PpTtee**	4 **PpttEe**	4 **ppTtEe**				
	4 **PPTtEe**							
	4 **PpTTEe**							
	4 **PpTtEE**							
	27 ‘ emerged ’	9 ‘ emerged ’	9 ‘ partially emerged’	9 ‘ enclosed ’	3 ‘ partially emerged ’.	3 ‘ tip emerged’	3 ‘ enclos-ed’	1 ‘ emerg-ed.’

Thus in the F_2 generation there are thirty-seven 'emerged', twelve 'partially emerged', three 'tip emerged' and twelve 'enclosed' plants. Since, in the study of the F_2, the first two classes had been amalgamated, we may expect the frequencies of the three groups to be in the ratio 49 : 3 : 12. The value of χ^2 calculated on this basis again shows that the observed frequencies do not fit well with the calculated ones. However, the study of the F_3 generation clearly indicated that the F_2 frequencies require correction, because, as has already been stated, in the previous generation the difficulty of accurate classification of the population as well as the dependence of the sheathed ear character for its expression on environment caused distortion of the frequencies.

It was found, for instance, that of the eight plants noted as 'tip emerged' in the F_2 only five were really so, two of them behaving in F_3 like 'enclosed' plants and one as a 'partially emerged' plant. Similarly, of the seventeen 'enclosed' F_2 plants as many as six proved to belong to the 'partially emerged' class. The frequencies corrected in light of the F_3 results are as follows :—

'Emerged' and 'partially emerged'	547
'Tip emerged'	37
'Enclosed'	117

The frequencies calculated on the basis of 49 : 3 : 12 ratio are 536·6, 33 and 131, respectively, for the three groups. The 'goodness-of-fit' test applied to the corrected figures is given below.

Goodness-of-fit test

	'Emerged' and 'partially emerged'	'Tip emerged'	'Enclosed'
$o - c$	10·4	4·0	14·0
$(o - c)^2$	108·2	16·0	196·0
$\frac{(o - c)^2}{c}$	0·20	0·48	1·50

$\chi^2 = 2·18$

Fisher's table of χ^2 [Fisher, 1932] shows that the probability of occurrence of the observed deviation is between 0·30 and 0·50. That is, the fit is good.

Let us now examine the different F_2 genotypes and see how far their behaviour conforms to the trihybrid scheme advanced. Of the forty-two F_2 plants selected twenty-four belonged to the group ‘ emerged ’ and ‘ partially emerged ’, five to the ‘ tip emerged ’ group and thirteen to the ‘ enclosed ’ group. We shall, in the following statement, represent these four groups by the abbreviations *Em*, *Pe*, *Tem* and *Ed*. The different phenotypes, their constituent genotypes, along with their formulæ and the observed and expected frequencies are given in Table I.

TABLE I

Genotypic classification of the selected F_2 plants of cross T 1 × T 131 and their observed and expected frequencies

Number and nature of F_2 parents	Constitution of F_2 genotype	Segregation in F_3	Frequency	
			Expected	Observed
24 *Em* and *Pe*	PPTTEE	Pure *Em*	0·49	
	PPTTEe	Do.	0·98 } 2·45	3
	PPTTee	Do.	0·49	
	ppttee	Do.	0·49	
	PPTtEE	3 *Em* : 1 *Pe*	0·98	
	PPTtEe	Do.	1·96 } 3·92	3
	PPTtee	Do.	0·98	
	PpTTEE	3 *Em* : 1 *Ed*	0·98	0
	PpTTEe	12 *Em* : 1 *Tem* : 3 *Ed*	1·96	0
	PpTtEE	9 *Em* : 3 *Pe* : 4 *Ed*.	1·96	2
	PpTtEe	37 *Em* : 12 *Pe* : 3 *Tem* : 12 *Ed*	3·92	6
	PpTTee	3 *Em* : 1 *Tem*	0·98	0
	PpTtee	10 *Em* : 3 *Pe* : 3 *Tem*	1·96	0
	PPttEE	Pure *Pe*.	0·49	
	PPttEe	Do.	0·98 } 1·96	2
	PPttee	Do.	0·49	
	PpttEE	3 *Pe* : 1 *Ed*	0·98	2
	PpttEe	1 *Em* : 12 *Pe* : 3 *Ed*	1·96	5
	Ppttee	1 *Em* : 3 *Pe*	0·98	1
5 *Tem*	ppTTee	Pure *Tem*	1·67	2
	ppTtee	1 *Em* : 3 *Tem*	3·33	3
13 *Ed*	ppTTEE	Pure *Ed*	1·08	
	ppTtEE	Do.	2·17 } 4·33	7
	ppttEE	Do.	1·08	
	ppTTEe	1 *Tem* : 3 *Ed*	2·17	1
	ppttEe	1 *Em* : 3 *Ed*	2·17	1
	ppTtEe	1 *Em* : 3 *Tem* : 12 *Ed*	4·33	4

It will be seen that a majority of the segregations was represented by the F_3 generation. With a greater number of F_3 cultures it would have been possible to get all the possible types of segregation. The frequencies in the segregating cultures may now be examined.

T

(1) Segregation into 3 *Em* : 1 *Pe* was represented by three F_3 cultures from F_2 genotypes of the constitution **PPTtEE, PPTtEe** or **PPTtee.** The frequencies observed were as follows :—

Culture No.	Segregation in F_3	
	Em	*Pe*
12	23	11
133.	46	17
178.	35	15
Total observed	104	43
Calculated on 3 : 1 basis	110·25	36·75

Deviation = 6·25 ; Probable error = 3·54

The fit is good.

(2) Segregation into 9 *Em* : 3 *Pe* : 4 *Ed* was represented by two F_3 cultures from F_2 genotypes of constitution **PpTtEE.** The frequencies observed were as follows :—

Culture No.	Segregation in F_3		
	Em	*Pe*	*Ed*
128	23	12	15
177	34	11	16
Total observed	57	23	31
Calculated on 9 : 3 : 4 basis . .	62·2	20·9	27·8
$\frac{(o-c)^2}{c}$	0·45	0·21	0·37

χ^2 = 1·03. The fit is good.

(3) Segregation into 37 *Em* : 12 *Pe* : 3 *Tem* : 12 *Ed* was represented by six F_3 cultures from F_2 genotypes of the constitution **PpTtEe.** The frequencies observed were as follows :—

Culture No.	Segregation in F_3			
	Em	*Pe*	*Tem*	*Ed*
4	15	17	2	12
37	27	18	3	11
41	37	22	4	34
100	58	41	2	4
109	22	25	5	10
188	41	14	4	11
Total observed . . .	337		20	82
Calculated on 49 : 3 : 12 basis . .	336·1		20·6	82·3

The fit is good.

The above frequencies can be reasonably expected from genotypes of the constitution **PpTTEe** as well. Such genotypes will segregate into 12 *Em* : 1 *Tem* : 3 *Ed* (Table I). Perhaps some of the above cultures have come from such genotypes rather than from genotypes of the constitution **PpTtEe**. Since the two segregations are very much alike it is difficult to separately identify the two kinds of genotypes. Considering the frequencies on 12 *Em* : 1 *Tem* : 3 *Ed* basis we get the following observed and calculated frequencies :—

	Em	*Tem*	*Ed*
Observed	337	20	82
Calculated	328·6	27·3	82·1
$\frac{(o-c)^2}{c}$	0·21	1·95	0·00

The fit is good.

(4) Segregation into 3 *Pe* : 1 *Ed* was represented by two F_3 cultures from F_2 genotypes of the constitution **PpttEE**. The frequencies observed were as follows :—

Culture No.	Segregation in F_3	
	Pe	*Ed*
43	44	19
154	31	15
Total observed	75	34
Calculated on 3 : 1 basis	81·75	27·25
Deviation ÷ Probable error	2·21	

The fit is good.

(5) Segregation into 1 *Em* : 12 *Pe* : 3 *Ed* was represented by five F_3 cultures from F_2 genotypes of constitution **PpttEe**. The frequencies observed were as follows :—

Culture No.	Segregation in F_3		
	Em	*Pe*	*Ed*
22	14	35	11
39	14	38	8
87	11	35	14
126	5	40	15
153	6	41	17
Total observed	50	189	65
Calculated on 1 : 12 : 3 basis	19	228	57
$\frac{(o-c)^2}{c}$	50·58	6·67	1·12

$\chi^2 = 58 \cdot 37$. The fit is bad.

However, f we combine the classes *Em* and *Pe* and consider the result on 13 *Em, Pe* : 3 *Ed* basis the fit becomes good, the ratio ' deviation : probable error ' being only 1·90.

(6) Segregation into 1 *Em* : 3 *Pe* was represented by one F_3 culture from an F_2 genotype of the constitution **Ppttee**. The frequencies observed were as follows :—

Culture No.	Segregation in F_3	
	Em	*Pe*
148	16	38
Observed	16	38
Calculated on 1 : 3 basis	13·5	40·5

The fit is good.

(7) Segregation into 1 *Em* : 3 *Tem* was represented by three F_3 cultures from F_2 genotypes of the constitution **PpTtee**. The frequencies observed were as follows :—

Culture No.	Segregation in F_3	
	Em	*Tem*
13.	11	50
150	13	33
165	13	33
Total observed	37	116
Calculated on 1 : 3 basis	38·25	114·75

The fit is good.

(8) Segregation into 1 *Tem* : 3 *Ed* was represented by one F_3 culture from an F_2 genotype of constitution **ppTTEe**. The frequencies observed were as follows :—

Culture No.	Segregation in F_3	
	Tem	*Ed*
137	17	35
Observed	17	35
Calculated on 1 : 3 basis	13	39
Deviation ÷ P. E.	1·9	

The fit is good.

(9) Segregation into 1 *Em* : 3 *Ed* was represented by one F_3 culture from an F_2 genotype of the constitution **ppttEe**. The frequencies observed were as follows :—

Culture No.	Segregation in F_3	
	Em	*Ed*
3	14	46
Observed	14	46
Calculated on 1 : 3 basis	15	45

The fit is good.

(10) Segregation into 1 *Em* : 3 *Tem* : 12 *Ed* was represented by four F_3 cultures from F_2 genotypes of the constitution **ppTtEe**. The frequencies observed were as follows :—

Culture No.	Segregation in F_3			
	Em	*Pe*	*Tem*	*Ed*
40	1	7	11	41
107. . . .	1	2	10	34
108. . . .	2	2	12	37
276. . . .	4	0	10	43
Total observed .	19		43	155
Calculated on 1 : 3 : 12 basis . . .	13·6		40·7	162·7
$\frac{(o-c)^2}{c}$ . . .	2·14		0·13	0·36

$\chi^2 = 2·63$. P = between 0·50 and 0·40. The fit is good.

The above analyses show that the F_2 and F_3 data can be interpreted completely on the trihybrid basis and the apparent contradictions in inheritance of the character reconciled. Similar conditions of inheritance were observed in the other crosses as well. Minute differences between the individuals of the same phenotype were very common and were possibly due to genes of minor importance.

The F_3 data presented above have been confined to forty-two cultures grown in 1934. The rest of the F_3 cultures grown in this year could not be examined in as much detail, although the broad facts about segregation were all that have been described. Further, although in 1932 as many as 516 F_3 cultures of the different *Sathi* crosses were grown and a plant-to-plant study of the sheathed ear character was made in many of the cultures a consolidated presentation of data was not possible till the 1934 studies were completed. One reason was that in the F_2 generations studied in 1931 the 'tip emerged' class was not given a separate identity, having been mixed with the 'enclosed' class on account of its approach to the latter class in appearance. Hence the relation between the F_2 generations and the following F_3 generations could not be completely and correctly judged in 1932. It was during the study of the F_3 cultures in 1932, that it became apparent that the 'tip emerged' class deserved a separate place. With the help of the experience gained a closer study of F_2 in 1933 and of F_3 in 1934 became possible.

The selections for the later generations were made mainly from the economic point of view, and the studies on sheathed ear already made helped greatly in the selection of desirable hybrids. In the F_6 generation grown in the rainy season of 1935 the majority of the population grown belonged to the 'enclosed' or the 'tip emerged' class, as these were the conditions aimed at. Incidentally it may be mentioned that many of the hybrids possess other desirable characteristics as well, and as such represent a successful progress of the work started eight years ago.

We have to acknowledge our indebtedness to the Imperial Council of Agricultural Research through whose generous grant it was possible to carry on the work described to a successful completion.

SUMMARY

Above is presented a study of the inheritance of a peculiarity in rice which determines the immunity of the paddy crop to the attacks of the rice fly, *Leptocorisa varicornis*. The peculiarity consists of an expansion of the leaf-sheath which keeps the ear covered throughout the life of the plant, thus preventing the fly from gaining access to the developing grains which would otherwise be eaten. The peculiarity styled as 'sheathed ear' in the paper, is present naturally only in a group of coarse-grained, poor-yielding rices called *Sathi*, but is absent from all the good and heavy-yielding rices. Crosses made between one of the *Sathi* types and some of the latter in 1929 have been studied, and the inheritance of the sheathed ear described. The 'emerged' condition was dominant in F_1. In F_2 four phenotypes were distinguishable, and named 'emerged', 'partially emerged', 'tip emerged' and 'enclosed'. For convenience of study the first two classes in F_2 were amalgamated, the resulting three classes being in the ratio 49 : 3 : 12. In F_3 diverse ratios were obtained and the inheritance appeared complicated and confusing, but the data have been satisfactorily explained on a trihybrid hypothesis. True-breeding hybrids have been obtained in F_6 some of which appear to be of great economic significance.

REFERENCE

Fisher, R. A. (1932). 'Statistical Methods for Research Workers', 4th Edition; Oliver and Boyd, London and Edinburgh.

APPENDIX

Segregation of sheathed ear in forty-two F_3 cultures in cross T 1 × T 131, studied in 1934

Culture No.	Character of F_2 parent	Frequencies in F_3				Segregation
		Em	*Pe*	*Tem*	*Ed*	
3	*Ed*	4	9	1	46	1 *Em* : 3 *Ed*
4	*Ed* (*Pe*)	15	17	2	12	12 *Em* : 1 *Tem* : 3 *Ed*
12	*Ed* (*Pe*)	23	11	..	..	3 *Em* : 1 *Pe*
13	*Tem*	6	5	42	8*	1 *Em* : 3 *Tem*
16	*Tem*	All *Tem*				Pure *Tem*
22	*Ed* (*Pe*)	14	33	2*	11	1 *Em* : 12 *Pe* : 3 *Ed*
37	*Em*	27	18	3	11	12 *Em* : 1 *Tem* : 3 *Ed*
39	*Pe*	14	38	..	8	1 *Em* : 12 *Pe* : 3 *Ed*
40	*Ed* (*Pe*)	1	7	11	41	1 *Em* : 3 *Tem* : 12 *Ed*
41	*Ed*	37	22	4	34	12 *Em* : 1 *Tem* : 3 *Ed*
43	*Pe*	..	44	..	19	3 *Pe* : 1 *Ed*
44	*Tem* (*Ed*)	..	6*	10	39	1 *Tem* : 3 *Ed*
86	*Em*	All *Em* and *Pe*				Pure *Em*, *Pe*
87	*Pe*	11	35	..	14	1 *Em* : 12 *Pe* : 3 *Ed*
100	*Pe*	58	41	2	4	12 *Em* : 1 *Tem* : 3 *Ed*
106	*Ed*	All *Ed*				Pure *Ed*
107	*Ed*	1	2	10	34	1 *Em* : 3 *Tem* : 12 *Ed*
108	*Ed*	2	2	12	37	1 *Em* : 3 *Tem* : 12 *Ed*
109	*Em*	22	25	5	10	12 *Em* : 1 *Tem* : 3 *Ed*
110	*Pe*	All *Pe*				Pure *Pe*
126	*Ed* (*Pe*)	5	39	1*	15	1 *Em* : 12 *Pe* : 3 *Ed*
128	*Pe*	23	12	..	15	9 *Em* : 3 *Pe* : 4 *Ed*
130	*Em*	All *Em*				Pure *Em*
133	*Pe*	46	17	..	..	3 *Em* : 1 *Pe*
137	*Ed*	..	4*	13	35	1 *Tem* : 3 *Ed*
148	*Pe*	16	38	..	..	1 *Em* : 3 *Pe*
150	*Tem*	10	3	33	..	1 *Em* : 3 *Tem*
151	*Ed*	All *Ed*				Pure *Ed*
153	*Pe*	6	41	..	17	1 *Em* : 12 *Pe* : 3 *Ed*
154	*Ed* (*Pe*)	..	31	..	15	3 *Pe* : 1 *Ed*
159	*Ed*	All *Ed*				Pure *Ed*
161	*Em*	All *Em*				Pure *Em*
165	*Tem*	10	3	32	1*	1 *Em* : 3 *Tem*

---

*Figures marked by asterisks represent individuals which should rightly have been included in some other group of phenotypes than the one in which they have been placed. It will be remembered that similar corrections were required in the case of the F_2 population as well.

Appendix—*contd.*

Culture No.	Character of F_2 parent	Frequencies in F_3				Segregation
		Em	*Pe*	*Tem*	*Ed*	
168	*Tem*	All *Tem*				Pure *Tem*
175	*Ed*	All *Ed*				Pure *Ed*
177	*Em*	34	11	..	16	9 *Em* : 3 *Pe* : 4 *Ed*
178	*Pe*	35	15	..	..	3 *Em* : 1 *Pe*
179	*Ed*	All *Ed*				Pure *Ed*
188	*Em*	41	14	4	11	9 *Em* : 3 *Pe* : 1 *Tem* : 3 *Ed*
198	*Ed*	All *Ed*				Pure *Ed*
207	*Tem* (*Pe*)	All *Pe*				Pure *Pe*
276	*Tem* (*Ed*)	4	10	..	43	1 *Em* : 3 *Tem* : 12 *Ed*

Phenotypes given in brackets in column 2 above represent the real character of the F_2 parent as revealed by the F_3 generation.

PRELIMINARY EXPERIMENTS ON THE MASS-PRODUCTION OF *TRICHOGRAMMA* PARASITES FOR CONTROL AGAINST SUGARCANE BORERS IN MYSORE

BY

T. V. SUBRAMANIAM

Entomologist to Government, Mysore, Bangalore

(Received for publication on 11th February 1936)

(With Plates X and XI)

INTRODUCTION

CONSIDERABLE attention has, in recent years, been devoted in the United States of America and West Indies to the biological control of borers of sugarcane by breeding one of their egg-parasites on a large scale in the laboratory and liberating them in the fields [Hinds and Spencer, 1928; Tucker, 1931]. Following the extensive work that has been going on abroad on the control of the sugarcane borer, *Diatraea saccharalis* Fab., by means of the parasitic wasp, *Trichogramma minutum* Riley, some preliminary attempts were made in this direction by the Mysore Agricultural Department in 1930 to study the possibility of utilising on similar lines this parasitic wasp which had been noticed to parasitise in the fields the eggs of the local sugarcane borer, *Agryria sticticraspis* Hmp. The results of these early trials are embodied in a short paper by the late Dr. Kunhikannan, Entomologist of the Agricultural Department, Mysore [Kunhikannan, 1931].

The object of the present paper is to set forth the results of (1) further trials made in the same direction, (2) observations on the life-history, host relations and general behaviour of the parasite under laboratory conditions; (3) observations on the value of the parasite in the cane field as a result of releases.

MATERIAL AND METHODS

(a) *Rearing Flour-moths*

As a result of repeated attempts the late Dr. Kunhikannan found that the *Trichogramma* parasite did not breed in the eggs of the moth, *Sitotroga cerealella* Oliv. although this appears to be the commonest host used in other countries. In Mysore the parasites have been found to breed very readily on the eggs of the *Pyralid* moth, *Corcyra cephalonica* Staint, a common pest of stored flours and cereals. For the studies on the utility of this parasite in Mysore the host eggs used were of this moth collected daily in sufficient numbers from the different stores and flour mills in Bangalore. The moths were also bred and multiplied in the breeding cupboards in the Entomological Laboratory. In Malaya, Miller and Pagden [1930] also utilized the eggs of this moth for the large-scale breeding of *Trichogramma minutum* for the control of the paddy stem-borers. Redman King [1934] utilized the eggs of *Ephestia kuhniella* in Ceylon for large-scale production of *Trichogramma evanescens* against the tea Tortrix, *Homona coffearea* Nietn.

With regard to the apparatus for rearing the host moth, trials with tin cylinders used by Kunhikannan did not give satisfactory results, for it was observed that a 20 good many of the caterpillars were unable to work their way up to the surface of the material in the cylinder for pupation and as a result many of the caterpillars which pupated in the lower layers of the material failed to emerge as healthy adults for prompt utilisation. In order, therefore, to facilitate the quick multiplication of the host, an arrangement with shallow moveable wooden trays with infested material in a cupboard was adopted (Plate X, fig. A). Most of the emerging moths which are generally found to settle on the sides of the door and the bottom of the tray are collected daily by means of small glass tubes. Methods of moth collection, simpler than those adopted in other countries are being investigated.

(*b*) *Egg collection from Flour-moths*

The apparatus used is similar to the one described by Kunhikannan [1931]. Two glass cylinders, five inches long and seven inches in diameter, open at both ends, were taken. One end of one of the cylinders was closed with wire-gauze (285 meshes to the square inch) and the other end covered with a lid of perforated zinc sheet. A thin card-board rolled into the shape of funnel is fixed inside the other cylinder so that the wide mouth of the funnel fits exactly into one of the open ends of the cylinder and its tapering end projects inside and remains within. The daily collection of moths is introduced into the first cylinder and placed over the funnel in the second cylinder with the wire-gauze bottom covering the mouth of the funnel (Plate XI, fig. A). The eggs laid by the moths during night time are found collected at the bottom of the funnel in the morning and a few eggs that are found attached to the wire-gauze are brushed into the funnel. The eggs in the funnel are then emptied on a sheet of paper; the scales and broken appendages of the moths found mixed up with the eggs are carefully removed, and the clean eggs emptied into a Petri dish. The moths lay on an average 23·64 eggs per day for four days, but after the fourth day the number gradually diminishes. The total daily collection of moths varies from 200 to 500 and of these 49·67 per cent are females. The average number of eggs produced per week in 1934 was 12,000, *i.e.*, ten egg cards containing 1,200 eggs per card approximately. The above average has been calculated from a nine months collection.

(*c*) *Parasite breeding*

The eggs of the moths thus obtained are evenly sprinkled over the surface of a card 1 in. × 3 in. smeared with a thin film of mucilage of prickly pear (*Opuntia*).

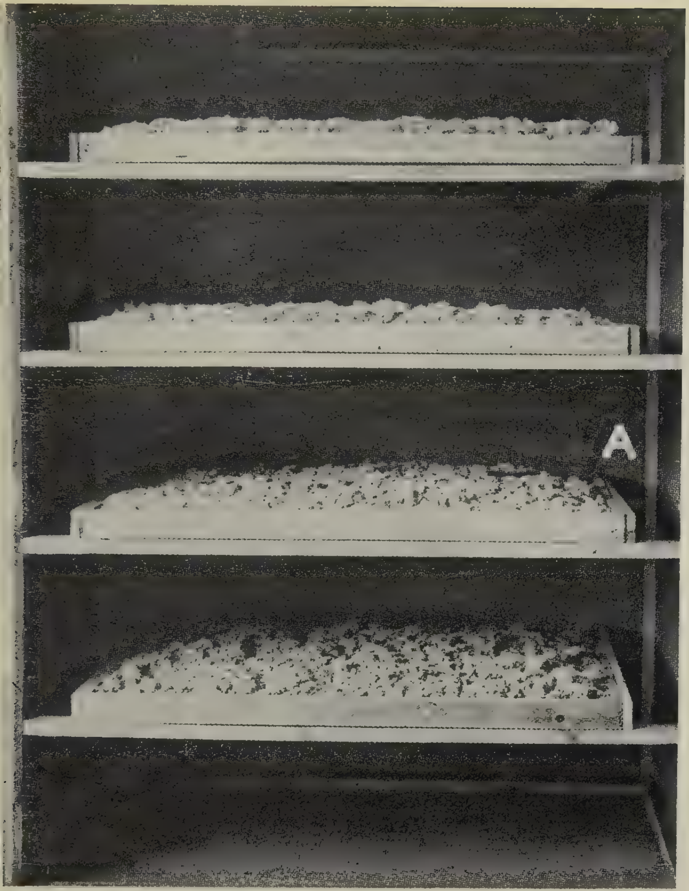


FIG. A. Interior view of a moth-breeding cupboard.

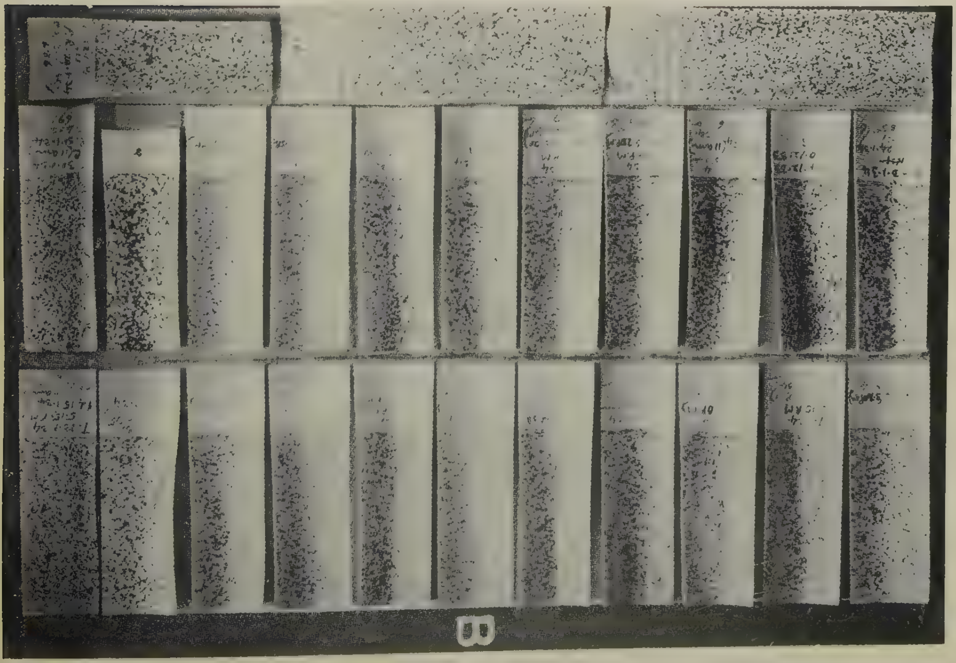


FIG. B. Parasitised Flour moth eggs in cards ready to be stored in a refrigerator.

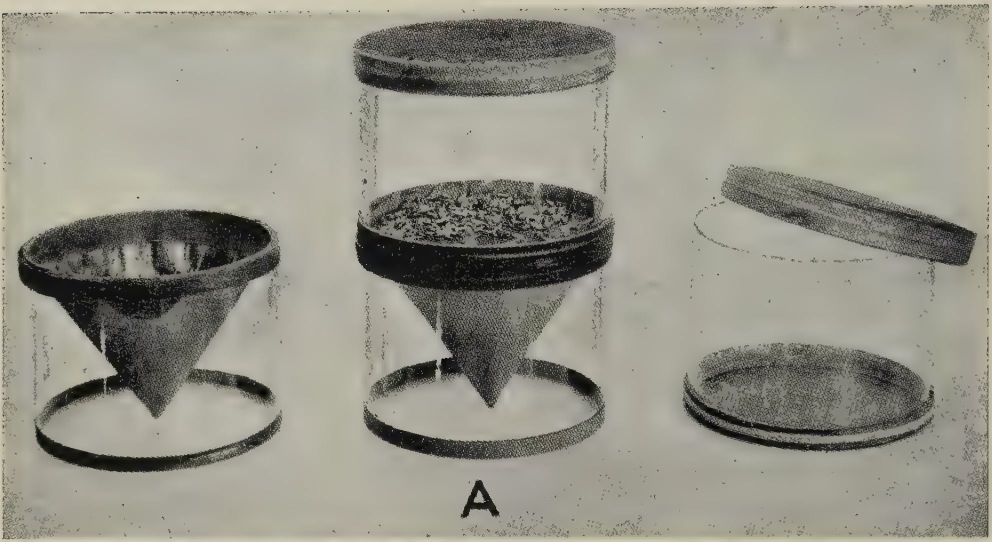


FIG. A. Glass cylinder used in collecting Flour-moth eggs.

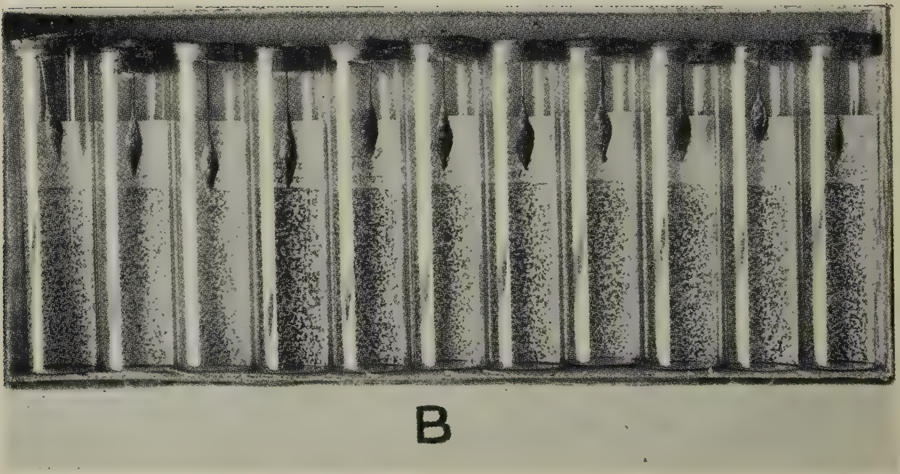


FIG. B. Tubes containing Flour-moth egg cards used in breeding *Trichogramma* parasites.

A space of ¾-in. is left at one end for noting details regarding dates of parasitisation, storage in cold chamber, etc. ; 1,000 to 1,200 eggs approximately were placed on each card. Each of these cards is introduced into small glass tubes, 1 in. × 4 in., containing about 1,000 parasites and the tubes are closed with corks to the inner end of which are attached small pins with bits of cotton-wool soaked in dilute honey or syrup to serve as food for the wasps. The cards are changed daily and fresh egg cards are put into the tubes (Plate XI, fig. B). The wasps are found to parasitise all the eggs in the cards effectively for three days. Although the wasps are able to continue parasitising the eggs in the fresh cards for five days, it has been found that the number of eggs parasitised gradually diminishes after three days and over fifty per cent of the eggs on the cards remained unparasitised on the fifth day.

LIFE-HISTORY AND HABITS OF THE PARASITE

The parasitised eggs on the cards can be recognised by their turning black in colour on the fourth day after parasitisation. From observations made on the emergence of thousands of wasps from a series of parasite cards it has been found that an average of 74·7 per cent are females and the rest males. Copulation takes place a short time after emergence. One female wasp was found to be alive for a maximum period of twenty-four days with a feed of dilute honey, but Hinds and Spencer [1928] state that they have not found that the supply of moisture or sweetened water in the Petri dish breeding-cages added appreciably to the length of the life of the wasps. Without any feed the wasps generally die in three days.

The average number of Flour-moth eggs parasitised by one wasp is 64·3. Out of the parasitised eggs on the cards an average of 69·07 per cent hatch and wasps emerge therefrom. Parthenogenetic reproduction takes place and the progeny in these cases is found to be all males and the developmental period the same as that when bred from fertilised eggs. The developmental period of the wasps in the parasitised Flour-moth eggs has been found to vary from seven to eleven days during the different months of the year. In the months of March and April when the maximum mean temperature ranges from 91·9°F. to 93·8°F. and the minimum 65·2°F. to 69·5°F. the developmental period of the wasps was found to vary from seven to eight days. From October to February when the maximum mean temperature ranges from 79·8° F. to 82·4°F. and the minimum 59·2°F. to 65·3°F., the developmental period varied from nine to eleven days. During the other months the developmental period varied from eight to ten days according to temperature variations. The parasite egg cards are stored in a refrigerator at a temperature ranging from 5°C. to 11°C. on the fifth day after parasitisation commences, *i.e.*, a day after the eggs turn black in colour (Plate X, fig. B). It is found that up to a period of thirty to thirty-five days in cold storage the normal average number of active wasps emerged which were capable of parasitising Flour-moth eggs. Any period longer than this resulted in a reduced per cent of emergence of the wasps. In one instance a few parasites in an egg card were found alive after remaining for about ninety days in cold storage, but they were inactive and weak and died shortly after emergence.

From the time the parasitised eggs are stored in a refrigerator the development of the parasite ceases and starts again soon after they are taken out and the total developmental period has been found to be normal. For example, if the life-cycle of the wasp is seven days and the eggs are stored in a refrigerator six days after parasitisation commences, the wasps are found to emerge one day after the eggs are taken out of the refrigerator irrespective of the number of days they have remained in cold storage, and if the eggs are stored four days after parasitisation commences the wasps emerge three days after the eggs are removed from the refrigerator.

SOME DIFFICULTIES IN PARASITE AND FLOUR-MOTH REARING

(1) The caterpillars of the Flour-moth hatch out in six days from egg-laying from some of the unparasitised eggs in the cards, construct tunnels of webbing among the parasitised eggs and eat them up. Many of the parasites which emerge get entangled in the webbing and are found dead before they could be utilised. Efforts are being made to find out some other host, the larvae of which have not this web spinning habit, for rearing the parasites.

(2) Other store pests, *viz.*, *Tribolium castaneum* Hbst. and *Tribolium confusus* Duv. are found to breed in large numbers on the flour in the moth-breeding cupboards. The Braconid parasites, *Microbracon brevicornis* Wsm. and *Rhabdepyris zeae* T. and W. are also noticed, perhaps parasitising the caterpillars in the cupboards, in large numbers. The emergence of the Flour-moth is found to be very much reduced when the above insects appear in large numbers. The moth-breeding cupboards are then cleaned thoroughly and fumigated with sulphur. The infested material is removed and a fresh stock of material is put in.

PARASITE UTILISATION AND PRELIMINARY RESULTS

Although preliminary trials in the release of the laboratory-bred parasites in sugarcane fields in different parts of the State were being made since 1931 it was not until 1933 that any intensive work and continued observations in this direction have been possible. The appearance of sugarcane borers in a serious form in young canes planted more or less throughout the year and extensive sugarcane cultivation around the newly-started Mysore Sugar Factory in Mandya, sixty miles from Bangalore, afforded very good opportunities for investigations on parasite releases and other methods of borer control. I have to mention here that the most serious of the sugarcane borers in Mysore is *Argyria sticticraspis* Hmp. and the greatest amount of damage is caused by this insect when the canes are from four or five weeks to about nine weeks old. Any control measures adopted then ensure the establishment of a very vigorous and healthy stand of canes ; much damage has not been found to be caused to the older canes by this borer.

The *Trichogramma* parasite colonization was made four times in small plots of not less than one acre in the Government Sugarcane Farm, Mandya, in 1933 and the results have been found encouraging. In one acre of planted canes between four and five weeks old in a twelve-acre block on the farm, the variety of cane being H. M. 320, the laboratory-bred *Trichogramma* release was commenced in the second week of October 1933. Weekly release of parasites emerging from six parasitic egg cards was continued for nine weeks, making a total of 45,000 parasites for the whole period. The initial parasitism of the *Argyria* borer eggs has been worked out month by month in the cane areas during 1933 and is given in Table I. Ten egg-masses of *Argyria* with a total of 600 eggs and five egg-masses with 500 eggs were picked up from different portions of the colonised area for percentage calculation, four weeks and ten weeks respectively after the commencement of parasite release. Four hundred and seventy-six eggs were found parasitised in the former and 462 in the latter, *i.e.*, 79·3 per cent and 92·4 per cent. The initial parasitisation for October was ten per cent and for December fifteen per cent. Thus an increase of 69·3 per cent in four weeks and 77·4 per cent in ten weeks has been obtained as a result of colonization.

TABLE I

Mean monthly percentage of natural parasitism of the eggs of Argyria sticticraspis *Hmp. by* Trichogramma minutum *Riley in 1933*

Months	Percentage
December to March	15
March to middle of May	5
Middle of May to July	6
July to September	2
September to December.	10

The actual weight of canes after harvest in the colonised and control area was found to be 16 tons 18 cwts. and 10 tons 4 cwts. per acre respectively, *i.e.*, an increase of 6 tons 14 cwts. per acre as a result of parasite colonization.

The parasite releases were made twice during the year 1934—once in the month of May and again in September—and the results obtained more or less confirm the figures given above for 1933. With regard to the extent of borer damage, the dead heart shoots appearing as a result of the *Argyria* borer attack in young cane plants have been recorded at the time of parasite release and at the termination of the colonization. These figures alone show a marked difference in the colonized area and the control. A statement showing particulars of the parasite releases in 1933-34 is given under Table II.

TABLE II

Particulars of Trichogramma *releases in young sugarcane fields during 1933, 1934 and 1935 with results*

Year and month	Area, age and variety of cane	Total parasites released and the period of release	Parasitisation of *Argyria* borer eggs at commencement of release of parasites	Parasitisation of *Argyria* after colonization		Increase due to colonization (per cent)	Number of "dead-heart" shoots at the time of colonization		Number of "dead-heart" shoots after parasite release		Weight of cane harvested		Increase
				Colonized	Check		Colonized	Check	Colonized	Check	Colonized	Check	
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8	(9)	(10)	(11)	(12)	(13)	(14)
October 1933 .	One acre, 4 weeks H. M. 320	45,000 in 9 weeks	10 per cent in both colonized and check	92·4 per cent (10 weeks after colonization)	15 per cent	77·4	Not recorded		Not recorded		16 tons 18 cwts.	10 tons 4 cwts.	6 tons 14 cwts.
May 1934 . .	Do. . .	26,500 in 6 weeks	Zero	76·8 per cent (6 weeks after colonization)	6 per cent.	70·8	462	430	432 (6 weeks after parasite release)	2,539	38 tons (Harvested for seeds when 9½ months old)	28 tons 10 cwts	9 tons 10 cwts.
September 1934 .	One acre, 3 weeks H. M. 320.	33,600 in 8 weeks	2·9 per cent.	80 per cent (8 weeks after colonization)	8 per cent	72	128	112	187 (8 weeks after parasite release)	542	57 tons 7 cwts.	50 tons 1 cwts.	7 tons 6 cwts.
April/May 1935 .	Umdihalli. One acre, 3 weeks. H. M. 320.	33,500 in 7 weeks	Zero .	64 per cent (7 weeks after colonization)	12 per cent	52	280	440	2,800	4,480	26·6 tons.	23 tons.	3·6 tons.
June/July 1935 .	Malligere Farm. 6 acres, 3 weeks. H. M. 320.	1,68,000 in 8 weeks	13·5 per cent (Two weeks after release started)	90 per cent (8 weeks after colonization)	...	76·5	160	Not recorded		...	40·45 tons.		
June/July 1935 .	Kothnahalli. 3 acres, 4 weeks H. M. 320.	1,02,000 in 5 weeks	Zero .	62·5 per cent. (5 weeks after colonization)	33·3 per cent.	29·2	80	120	2,960	5,280	24 tons	19·7 tons.	4·3 tons.
July/August 1935	Kanali "A" Farm 2 acres, 6 weeks H. M. 320.	72,000 in 5 weeks	No eggs found	...	Not recorded		1,320	Not recorded	2,560	Not recorded	43 tons	39 tons	4 tons
	Kanali Farm "B" 2 acres, 4 weeks. H. M. 320.	95,000 in 5 weeks	3·3 per cent	62·6 per cent (5 weeks after colonization)	Not recorded	59·3	640	Not recorded	3,040	Not recorded	*	*	

*Crop not yet harvested. But estimates show a 4-ton increase per acre.

During 1935 *Trichogramma* parasites were released in the ryots' fields in several localities round about Mandya. A statement showing the results of the parasite releases in those areas during 1935 is also given under Table II.

CONCLUSION

The work done so far has been only of a preliminary nature. The good results obtained in the borer control in small areas of sugarcane by *Trichogramma* parasite releases resulted in the establishment of a parasite laboratory in the centre of the largest cane-growing area in the State, *viz.*, Mandya, for the large-scale production of *Trichogramma* parasites and the study of sugarcane insects and their parasites.

REFERENCES

Hinds, W. E. and Spencer, H. (1928, 1). *International Congress of Entomology, Transactions II*, p. 1032.

08 ——————— (1928, 2). *Jour. Econ. Ent.* **21**, 273.

Kunhikannan, K. (1931). *Jour. Mysore Agric. and Expt. Union* **12**, 57.

Miller, N. C. E. and Pagden, H. T. (1930). *Malayan Agric. Jour.* **18**, 334.

376 Redman King, C. B. (1934). *Tea Quart. Jour.* **7**, 15.

98 Tucker, R. W. E. (1931). *Trop. Agric.* **8-11**, 283.

IMPORTANCE OF THE STUDY OF INSECT ECOLOGY IN APPLIED ENTOMOLOGY

BY

TASKHIR AHMAD, Ph.D. (Cantab.)

Assistant Entomologist, Imperial Institute of Agricultural Research, New Delhi

(Received for publication on 10th June 1936)

(With three text-figures)

I. Introduction

Insect ecology as we understand it today is the study of the behaviour of an insect in relation to its environment. Of the various physical factors of environment, temperature, humidity and light are the most important, while food, animal friends (hyper-parasites) and enemies (parasites and competitors) comprise the chief biological factors of environment.

It has long been known that physical environment exerts profound influence on the activities of living organisms but exact evaluation of such influences had not been possible until accurate investigations on the influence of individual factors, *e.g.*, temperature, humidity, etc. were commenced. For instance, studies at controlled temperatures yield fundamental information regarding the threshold of development, *i.e.*, the temperature below which no development is possible, and the upper vital limit or the temperature above which viability is seriously impaired. They also give an exact idea of the rate of development, percentage mortality, and power of reproduction and multiplication between the two limits mentioned above. The same is true of the factor humidity. Such information obtained from the study of individual factors can in turn be interpreted to apply to a combination of conditions prevailing in nature and thus help us in forecasting future outbreaks of particular species under known meteorological conditions. It has therefore become no longer sufficient for applied entomologists to watch the development and life-histories of insects under uncontrolled conditions alone: the importance of the study of injurious or beneficial insects in controlled environments cannot be too strongly emphasized.

Rapid progress has been made in Europe and America in investigating insects of economic importance under accurately controlled conditions of temperature and moisture. Recent works of Schnaur [1929], Janisch [1933], Zwölfer [1931-34] and others in Germany admirably show the importance and utility of correlating insect multiplication with environmental conditions. In U. S. A. Shelford [1927] as a result of his investigations, has given tables of developmental units for the notorious Codling moth, from which it has been possible to forewarn the farmers of different localities regarding the approximate dates of appearance of the pest.

In India, however, the life-histories and habits of insect pests of crops have been hitherto investigated, mostly under uncontrolled conditions of environment. Some of the notable exceptions are the ecological studies by Husain and others on the cotton Pink Bollworm, *Platyedra gossypiella* S. [Husain *et al.*, 1931-34] and the desert locust, *Schistocerca gregaria* F. [Husain *et al.*, 1936] carried out in the Punjab during the last few years. The uncontrolled conditions under which most of the work has been done are so varying and complex that they are not capable of exact measurement or expression. Such studies while furnishing some preliminary knowledge about the species under investigation cannot help us much in anticipating the behaviour of the species in the various regions of India having different climatic conditions. Moreover, owing to the fact that exactly similar micro-climatic conditions seldom recur, nor are they capable of exact duplication artificially, the results obtained by one worker are not susceptible of confirmation or contradiction even at the same locality, much less in other parts of the country or the world abroad. Naturally therefore the results of such work are of very limited application. It is therefore not too early for entomologists in India to pay attention to this fundamental aspect of research on insect pests.

To illustrate the practical importance and utility of such studies, the ecological observations made by the writer on the notorious Flour-moth, *Ephestia kühniella* and its parasite, *Nemeritis canascens* [Ahmad, 1936, 1], the rate of development and viability of the eggs of the migratory locust, *Locusta migratoria*, and the pupae of certain flies [Ahmad, 1936, 2] are briefly reviewed in the following pages.

II. INFLUENCE OF PHYSICAL ENVIRONMENT

1. On the power of reproduction

The biological equilibrium of any living organism in nature is brought about by the interaction of two forces : the biotic potential or the inherent power of reproduction of the organism and the resistance of the environment to its multiplication. The net result is a certain intensity of population which remains constant so long as the difference of the two forces remains unchanged. All the three foregoing conditions can be theoretically evaluated by studying the organism under controlled ecological conditions. The average number of eggs laid per female affords a measure of the biotic potential of the species. As all the eggs laid do not develop into adults, the sterility of the eggs laid and the mortality of the offspring during the various stages of development, constitute a measure of the environmental resistance. Subtracting the latter from the former and accounting for the sex ratio, *i.e.* the percentage of females to males, we get what is technically termed as the effective reproductive potential of the insect. This considered in conjunction with the rate of development, or in other words the number of generations in a year, helps us to predict with fair accuracy the intensity of population of a species after any given period of time.

The effective reproductive potential of *Ephestia kühniella* as worked out from its biotic potential and environmental resistance under different conditions is given in Table I.

TABLE I

The effective reproductive potential of Ephestia kühniella *under different conditions*

Temp. °C	Saturation deficiency (mm.)	Average No. of eggs per female	Percentage sterility in eggs	No. of fertile eggs per female	Percentage viability of eggs	No. of larvae hatched per female	Percentage larval and pupal mortality	No. of moths in nd generation per female	Effective reproductive potential *
18 .	3	196	14	169	84·2	142	15·2	120	64·80
	9	188	21	149	82·4	123	35·8	79	42·66
	14	142	32	97	74·6	72	63·4	26	14·04
23 .	3	212	14	182	85·8	156	7·2	145	78·30
	9	215	18	176	82·0	144	28·8	103	55·62
	14	205	22	160	72·5	116	60·0	46	24·84
	21	171	34	113	67·5	76	71·4	22	11·88
30 .	3	204	60	82	75·1	62	24·4	47	25·38
	9	165	73	45	76·3	34	28·9	24	12·96
	14	110	78	24	74·0	18	62·7	7	3·78

* A sex ratio of 54 per cent females has been used [after Payne, 1933].

It will be observed that though the fecundity of the females, *i.e.*, the total number of eggs laid at 30°C. and 3 mm. saturation deficiency is very much the same as that at 23°C. and 3mm. saturation deficiency, the effective reproductive potential is markedly reduced in the former case owing to increased environmental resistance. The effective reproductive potential is maximum at 23°C. and saturation deficiency of 3 mm., which thus represent the most optimum conditions for the reproduction and multiplication of this pest.

2. *On the rate of development*

(*i*) *Effect of constant temperature.*—Of all the ecological factors temperature exerts the most profound influence on the development of insects. Higher the temperature, within certain limits, quicker is the development (Tables II and III).

The rate of development at different constant temperatures can be best represented graphically by plotting the reciprocal of duration of development against temperature. The course of this, the index of development approximately follows a straight line. The point where the line cuts the temperature axis represents the threshold of development. The period plotted against temperature gives the hyperbolic curve. The rate of development of the eggs of *Locusta migratoria* and pupae of *Muscina stabulans* and *Calliphora erythrocephala* at different constant temperatures as shown in Figs. 1-3 illustrates these points.

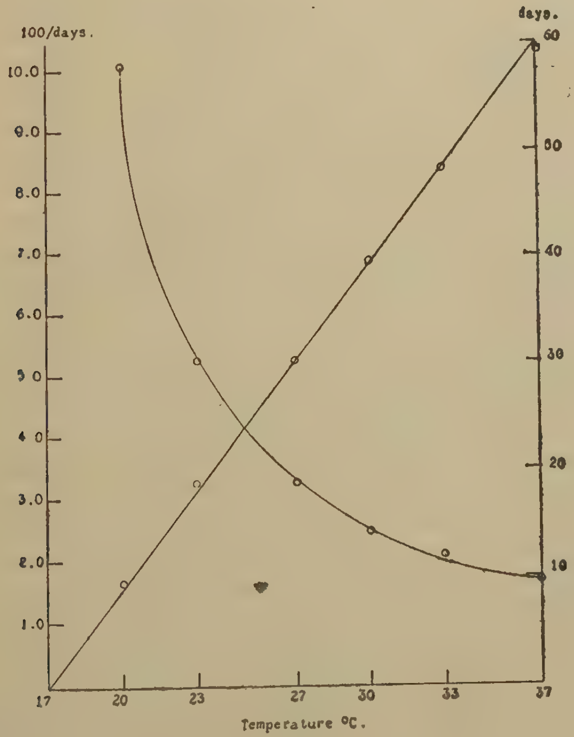


Fig. 1. The index of development and hyperbolic curve for the incubation period of *Locusta migratoria* eggs.

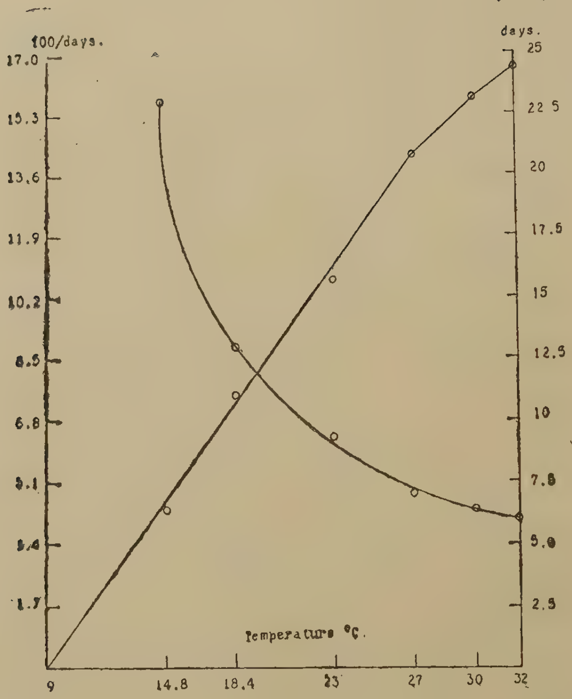


Fig. 2. The index of development and hyperbolic curve for the pupal period of *Muscina stabulans*.

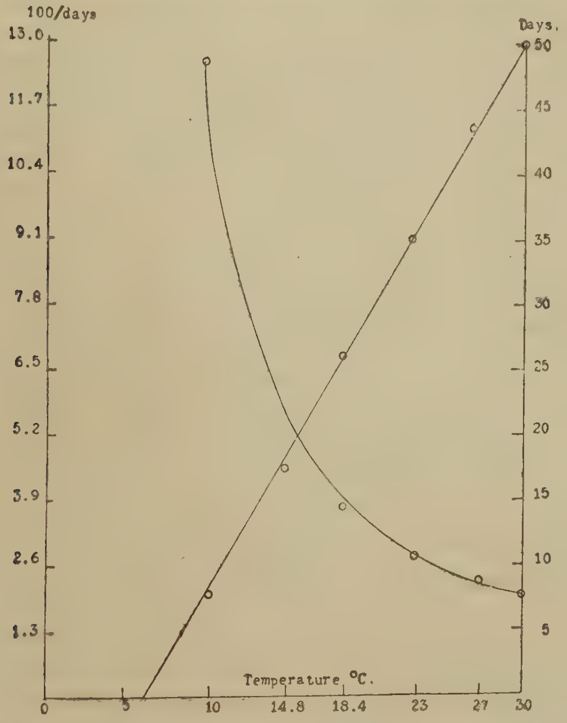


Fig. 3. The index of development and hyperbolic curve for the pupal period of *Calliphora erythrocephala.*

The lines of index of development are almost straight and the theoretical thresholds of development in the three cases lie at 17°C., 9°C. and 6°C. respectively. This fact alone reveals fundamental differences in the habits and habitats of the three species and indicates probable regions of their distribution. The first is undoubtedly a tropical insect and will not be able to thrive in any country where a temperature lower than 17°C. prevails for any considerable length of time. This indicates that cold, temperate countries should remain almost completely free from this locust. Taking temperature alone into consideration greater part of India which is periodically invaded by *Schistocerca gregaria,* an insect with almost similarly high threshold of development, has little possibility of becoming a permanent habitat for the pest. It may be mentioned however that temperature is not the only factor determining the regions of distribution of a species : abundant supply of food, suitable humidity and other factors may offer protection and assist the insect in tiding over periods of reasonably unfavourable temperatures.

The Blow-fly, *C. erythrocephala,* has a very low threshold of development and is probably, therefore, an inhabitant of cooler regions. At temperatures above

33°C. it suffers very high mortality. This temperature therefore represents the upper vital limit for this species although for a tropical insect like locust this is just an optimum condition for development. It is evident that in many parts of India where this temperature occurs sufficiently long during summer there is little possibility of the breeding of such an insect. Determination of upper vital limit has further practical utility in the heat-sterilization of stored grain insects and therefore merits special attention.

(*ii*) *Effect of fluctuating temperature.*—While studies on the influence of constant temperatures yield invaluable information regarding the behaviour of an organism to its environment, it must be remembered that in nature it is the fluctuating temperature to which most of the insects are exposed and the latter may exert an influence different from what would be expected on the basis of constant-temperature studies. In the case of several species it has been found that insects develop faster under varying temperatures than under constant temperatures. The cases of the Codling-moth [Shelford, 1927] and the larvae of Cutworm, *Porosagrotis orthogonia* [Cook, 1927] may be quoted as examples. Peairis [1927], as a result of his observations on the Blow-fly, *C. vomatoria,* suggested that the apparently accelerating effects of alternating temperatures should be regarded as the normal course and that the constant temperatures not being in line with the natural environment of most insects produce retarding effect on development. All insects however are not exposed to fluctuating temperatures in their natural habitats : stored grain insects, wood borers and subterranean species like termites are better adapted to a more or less constant environment. This may be the reason why in the case of the eggs of the Mediterranean Flour-moth, constant temperatures produced more rapid development than the alternating temperatures [Hase, 1927]. Again, certain species of insects, *e.g., Melanoplus mexicanus,* pass the winter in the egg stage. The immature stages of this species are exposed in nature to long spells of cold. This perhaps explains why Parker [1930] noticed a striking acceleration in the development of the eggs of this insect at high temperature following an exposure to low temperature. The longer was the period of exposure to low temperature the greater was the acceleration produced.

The writer's experiments on the development of eggs of *Locusta migratoria* and pupae of *Calliphora erythrocephala* and of *Muscina stabulans* at constant and alternating temperatures showed that the response of different species was very likely determined by the natural environment of the insect concerned.

In the case of *L. migratoria* eggs it was observed that fresh and one-fourth developed eggs exposed to a low temperature of 5°C. for one to eight days and then kept at a constant optimum temperature developed faster than those kept at the constant optimum temperature throughout. A rather long exposure of about sixteen days to 5°C. however, produced adverse effect on development. Similarly if more than half-developed eggs were exposed to 5°C. for one to eight days the rate of development at subsequent optimum temperature was retarded. But eggs kept daily for sixteen hours at an optimum temperature and for the remaining eight hours at 5°C. showed a marked acceleration in development. It was further evident that longer was the period of continuous exposure to cold and higher was the temperature of incubation, more adversely was the viability of the eggs affected.

It may be mentioned here that in this species the winter is passed in the adult stage and egg-laying begins in spring as soon as the weather gets warmer. In nature the eggs are therefore not exposed to any long spells of cold but are adapted to warm days alternating with fairly cool nights. This explains why in these experiments the viability of the eggs was lowered and their development retarded by a continuous long exposure to cold and that on the other hand daily alternation of temperatures stimulated the development.

The threshold of development of the Blow-fly pupae is rather low being about 6°C. Under natural conditions the pupae are adapted to long exposures to low temperature. For this reason it seems that their rate of development was definitely accelerated at alternating temperatures with single long exposure as well as daily exposures to a low temperature of 5°C., irrespective of the stage of development at which they underwent this exposure to cold. The longer was the period of exposure to cold, the greater was the acceleration in development at the subsequent optimum temperature. The acceleration showed a decline as the subsequent optimum temperature rose and was maximum at 23°C. This, therefore, represents the most optimum condition of temperature producing maximum acceleration.

Pupae of *Muscina stabulans*, on the other hand, registered very little acceleration in development at different temperatures following an exposure to 5°C. Pupae showed a susceptibility to low temperature and particularly the fresh ones suffered high mortality. In the adult stage the Stable-fly, *M. stabulans*, lives inside houses and therefore undoubtedly undergoes very little fluctuation of temperatures although immature stages are known to exist in a great variety of environment. In these experiments the development of the pupal stage was little affected by alternating temperatures, while prolonged exposures to cold appreciably increased the mortality.

From a study of the three insects detailed above it is evident that the response of insects to the influence of constant and alternating temperatures varies with different species and is most probably determined by the natural environment of the species concerned : each insect pest therefore merits independent critical ecological investigation to enable entomologists to forecast their multiplication and intensity of population and thus be fore-armed for the control measures.

(*iii*) *Effect of moisture.*—There is considerable amount of literature with almost empirical deductions regarding the influence of moisture on insect activities. Not infrequently mass outbreaks of certain species have been ascribed to dry or wet conditions prevailing during the periods preceding such outbreaks.

One might give an instance of the hitherto controversial views held about humidity requirements of *Ephestia kühniella*, an important pest of stored grains. Voukassovitch [1929] finding normal hatching of eggs under dry conditions concluded that dry environment was more favourable to this insect. She did not take into consideration the larval stage which is highly susceptible to environmental moisture. Speicher [1931] on the other hand observed that dry atmosphere and dry food diminished the size of *E. kühniella* larvae and slackened their development. Studies carried out by the writer under controlled conditions of moisture and heat have revealed the exact mositure requirements of this insect. It appears (Table I) that a moist atmosphere with a saturation deficiency of 3 mm. is the most favourable for its development and reproduction.

TABLE II

The rate of development and viability of C. erythrocephala *pupae at constant temperatures and saturation deficiencies*

Temp. °C.	Pupal period				Per cent viability			
	3 mm.	9 mm.	14 mm.	21 mm.	3 mm.	9 mm.	14 mm.	21 mm.
10·5	49·04	48·58	..	..	16·0	65·0	..	..
14·8	23·53	22·10	..	..	53·8	62·2	..	..
18·4	14·61	14·58	14·92	..	92·0	94·9	78·4	..
23·0	11·01	10·92	11·00	11·24	73·1	91·1	69·0	59·2
27·0	8·85	8·81	8·86	9·03	26·1	50·0	39·8	33·6
30·0	7·87	7·81	7·85	7·83	7·8	29·5	25·5	19·4

TABLE III

The rate of development and viability of Muscina stabulans *pupae at constant temperatures and saturation deficiencies*

Temp. °C.	Pupal period				Per cent viability			
	3 mm.	9 mm.	14 mm.	21 mm.	3 mm.	9 mm.	14 mm.	21 mm.
14·8	23·04	..	..	..	8·0	0·0	..	..
18·4	13·20	13·22	13·83	..	80·0	80·6	60·0	..
23·0	9·35	9·41	10·13	10·75	88·9	77·3	58·7	16·9
27·0	7·03	7·05	7·12	7·33	85·9	80·0	61·8	23·5
30·0	6·35	6·36	6·71	6·75	80·0	72·6	47·5	5·0
32·0	5·99	6·21	6·23	..	43·0	31·1	24·0	0·0

In Tables II and III are given the rate of development and percentage viability of *C. erythrocephala* and *M. stabulans* pupae under different conditions of moisture, *viz.*, saturation deficiencies of 3, 9, 14 and 21 mm. at various constant temperatures. It will be noticed that at any constant temperature the rate of development and percentage viability are maximum at a constant degree of mositure, *viz.*, 9 mm. saturation deficiency in the case of Blow-fly pupae and 3 mm. saturation deficiency in the case of *Muscina* pupae. These are therefore the most optimum conditions of moisture at the specific temperatures for the two species. Both development and viability show a fall when the saturation deficiencies deviate from the respective optima. It is further clear that the Blow-fly prefers a drier environment than *Muscina*.

Thus, in addition to an optimum temperature each insect species has a particular optimum condition of atmospheric moisture, a condition which may be dry as compared to the optimum of one insect and yet moist in relation to that of another. For instance the optimum moisture requirement of *C. erythrocephala* pupae (a saturation deficiency of 3 mm.) is more moist in comparison to the optimum moisture requirement of *M. stabulans* pupae (saturation deficiency of 9 mm.) and is yet dry when compared with the mositure requirement of *Locusta migratoria* eggs (saturation deficiency of 0 mm.). It will be realised therefore that 'dry' and 'moist' are only relative terms and are therefore sometimes very inadequate and even misleading in expressing the moisture requirements of various insects.

III. EFFECT OF BIOLOGICAL ENVIRONMENT OR HOST-PARASITE COMPETITION

The relations of insect hosts and their parasites, often intricate and difficult to understand in natural environment become intelligible if a critical ecological study is made thereof.

Reverting to the figures of reproductive potential of *Ephestia kühniella* (Table I) and comparing them with those of its parasite *Nemeritis canascens* (Table IV) we find that the reproductive potential of the host is markedly susceptible to high temperature and high saturation deficiency. *Nemeritis canascens*, being endoparasitic, its reproductive potential is neither affected by saturation deficiency nor appreciably by temperature.

TABLE IV

The reproductive potential of Nemeritis canascens *at constant temperatures*

Temperature °C.	Reproductive potential
15	3·3
18	37·0
23	38·7
30	34·0

W

At high temperature, *viz.*, 30°C. the parasite can always overtake the host on account of its higher reproductive potential and quicker development. At 23°C. the parasite can complete two generations in the same time in which the host completes one, whatever the humidity of the air, and would therefore annihilate the host in spite of its lower reproductive potential. At or below 18°C. the reproductive potential of parasite falls below that of the host and its rate of development also slackens so that there is no possibility of the parasite overtaking the host. The threshold of development of the host lies between 8°-10°C., that of the parasite between 12°-15°C., so that while the host will continue to develop between 10°-15°C., the parasite cannot do so. Thus, on the whole, high temperatures favour the parasite, low temperatures favour the host.

The influence of light on *E. kühniella* is generally exactly opposite to that on its parasite, they being negatively and positively heliotropic respectively. But the parasite seems to have adapted itself to the habit of the host as it is negatively heliotropic after feeding.

It will be readily realized that study of this nature is a necessary preliminary for assessing the utility or otherwise of a parasite for the control of a host pest under different ecological conditions. Such a study also gives the essential information about the most optimum conditions of temperature, humidity, light, etc., for the artificial propagation and mass-multiplication of a parasite and defines the viability zones and indicates regions climatically unsuitable for them.

In the end I must express my grateful thanks to Dr. H. S. Pruthi, Imperial Entomologist, for appreciating the necessity and welcoming the initiation of ecological studies at the Imperial Institute of Agricultural Research. It is at his suggestion that this semi-popular article has been written. The new Entomological Laboratory at Delhi is being fully equipped for such work and it is hoped that our important crop pests will be soon investigated as outlined herefore.

SUMMARY

The importance of the study of insect ecology in reference to applied entomology is emphasized and illustrated by the author's recent work on the influence of controlled ecological factors on some insect species. The effective reproductive potentials of *Ephestia kühniella* and its parasite *Nemeritis canascens* under different conditions of temperature and moisture have been worked out and compared. The rates of development of eggs of *Locusta migratoria* and pupae of *Calliphora erythrocephala* and *Muscina stabulans* at constant temperatures and saturation deficiencies are shown graphically and optimum requirements of moisture and temperature for each species are indicated. A critical ecological study of our important crop pests is urged and the utility of such studies in forecasting outbreaks of insect pests in the future is pointed out.

REFERENCES

Ahmad, T. (1936, 1). *Jour. Anim. Ecol.* **5**, 67-93.

——————(1936, 2). *Proc. National Inst. Sci. India* **2**, 67-91.

Cook, W. C. (1927). *Jour. Econ. Ent.* **20**, 769-82.

Hase, A. (1927). *Arb. biol. Anst. Land. Forstw. Berlin* **15**, 109-33.

Husain, M. A., Bindra, S. S., Ladha Ram, Ganda Ram and Dev Raj (1931). *Ind. Jour. Agric. Sci.* **1**, 211.

Husain, M. A., Khan, M. H., and Ganda Ram (1934). *Ind. Jour. Agric. Sci.* **4**, 261.

Husain, M.A., and Ahmad, T. (1936). *Ind. Jour. Agric. Sci.* **6**, 188.

Janisch, E. (1933). *Arb. biol. Anst. Land. Forstw. Berlin* **20**, 269-90.

Parker, J. R. (1930). *Univ. Montana Agric. Expt. Stn. Bull.* **223**, 1-132.

Payne, N. M. (1933). *Biol. Bull.* **65**, 187-205.

Peairis, L. M. (1927). *Bull. West Virginia Univ. Agric. Expt. Stn.* **208**, 1-62.

Schnaur, W. (1929). *Z. Angew Ent.* **15**, 565-627.

Shelford, V. E. (1927). *Bull. Illinois Nat. Hist. Surv.* **16**, 307-440.

Speicher, B. R. (1931). *Proc. Pennsyl. Acad. Sci.* **5**, 79-82.

Voukassovitch, P. (1929). *C. R. Soc. Biol. (Paris)* **100**, 62-4.

Zwölfer, W. (1931). *Z. Angew. Ent.* **17**, 475-562.

——————(1933). *Z. Angew. Ent.* **20**, 1-50.

——————(1934). *Z. Angew. Ent.* **21**, 333-84.

THE COLD STORAGE OF NAGPUR ORANGES (*CITRUS AURANTIUM*)

BY

G. S. CHEEMA, D. Sc., I. A. S.
D. V. KARMARKAR, Ph. D.

AND

B. M. JOSHI, M. Sc.

(Received for publication on 27th October 1936)

Refrigeration by mechanical means as a method of storing fruits, vegetables and meat is not so widely practised in India as in the United States and the European countries. The introduction of refrigeration in India is comparatively recent. Its importance, however, is now being realized by the fruit and meat trade. During the last four or five years, several cold storage plants have been installed in Bombay and other important cities, where a good deal of imported fruit and meat are stored.

The Imperial Council of Agricultural Research, India, realizing the growing importance of cold storage in the sphere of the world trade, installed an experimental cold storage plant in Poona in 1934 in order to investigate problems relating to the cold storage requirements of important Indian fruit and vegetables. The principal fruits on which research work has been carried out so far are the mango and the Nagpur orange (Santra).

The utility of low temperature for the storage of citrus fruit has been known for many years. It has always been a subject for intensive investigation in orange-growing and exporting countries. During recent years, the export trade in oranges from Australia, South Africa and the United States of America has rapidly increased and considering the time required for transport and the occurrence of heavy wastage due to high temperatures in transit, refrigeration has been found to be essential.

Rose and others [1933] found that oranges could be held for one or two months at 32° F. without any deterioration in appearance or flavour. Among California varieties, the Valencia orange was found to store best. The investigations of Adam [1923] with Washington Navel and Valencia oranges indicated that 34°F. is the most favourable storage temperature, taking into consideration both keeping quality and freedom from rotting. Tindale [1927] considered 37°F. to be the most suitable temperature for Valencia late oranges as a fluctuation of 3° F. either way would not be attended with harmful results. Guerrero [1927] in his storage studies with native oranges, known locally as the *naranghita* and the *cahit*, found that under proper conditions, *cahit* oranges could be held for a considerable period. The *naranghita* yellow orange, a tangerine, proved to possess poor keeping qualities. Chen [1934] kept several types of oranges in storage at a temperature of 5° to 12° C. and a relative humidity of 90 per cent or over. Thick-skinned oranges with high acid and solid content were considered to possess good keeping qualities. Loose-skinned oranges which were initially low in acid became insipid in flavour after four months. After five months the pulp was dried and shrunken, although externally the peel appeared normal. Observations of Williams [1935] indicated that on all occasions, oranges stored well at 38°F. to 40°F. at a humidity of 84 per cent. Below 38° F. brown spotting of the skin occurred and above 40° F. development of blue mould took place. Friend and Bach [1932] observed that Valencia oranges kept very satisfactorily at 32° F. even for so long a period as twenty weeks and the maturity of fruit was less of a factor in keeping than environment, particularly the soil and the rains occurring during harvest. Fruits harvested slightly past prime kept poorly.

It was shown in experiments [Leonard and Baker, 1934] carried out in 1933 at the Low Temperature Research Station, Imperial College of Tropical Agriculture, Trinidad, that the King orange or Giant Mandarin (*Citrus nobilis*) were held at 45° F. for sixty days with negligible wastage. At 50° F. fruits picked ripe, though still very attractive externally had lost all traces of acidity and their flavour, the juice being insipid, sweet and watery. Green fruits became ripe at 45° F. Even at this temperature fruit picked ripe had lost all its flavour. Leonard [1936] in a recent memoir on the storage of Trinidad citrus fruits has recommended 45° F. for Valencia oranges and 40° F. for King oranges as suitable for export. Young and Read [1931] carried out experiments on the preservation of citrus fruits for the Council of Scientific and Industrial Research of Commonwealth of Australia, with a view to lengthening the storage life of oranges. They found that Valencia oranges could be kept in good condition for three to four months at 38° F. without practically any loss. They also showed that Navel oranges kept best at 45° F. Navel oranges at 32° F. acquired a bitter taste after about five to six weeks. After eleven weeks, this was much more marked and the fruit showed a collapsed appearance, the skin becoming dull and discoloured. At 50° F., however, the fruit showed no signs of this appearance after fifteen weeks and had a total wastage of five to six per cent whilst the flavour was quite normal. Barker [1935] of Cambridge reported that it is customary to carry oranges at a temperature of about 40° F., since browning and pitting of the skin may occur at lower temperatures. Recently, a fully representative Advisory Technical Committee [1935] has been formed including representatives of the Council of Scientific and Industrial Research of Australia to control the investigations on preservation of citrus fruits. The programme includes a comprehensive scheme of investigation into the preservation of Navel oranges.

The observations detailed above conclusively show the useful application of low temperatures for prolonging the storage life of oranges. It is also evident that a number factors such as variety, maturity of fruit at the time of picking, and climatic and soil conditions are important and exercise considerable influence in determining the keeping quality of fruit in cold storage. It was, therefore, considered essential that the cold-storage requirements of the Nagpur orange should be studied in detail to help the prospective export trade. The investigations were started in March 1935 and the results obtained during the 1935 season are reported in this paper.

PLAN OF EXPERIMENTAL WORK

The oranges used in these experiments were obtained directly from Nagpur. They were in railway transit for two days. On arrival, they were carefully examined, fruits showing any blemish or puncture in the skin being rejected from the experiment. The selected oranges were again graded into three lots according to the stage of maturity as (1) green, (2) turning and (3) yellow or fully ripe.

The fruits of the three grades were stored at the four temperatures, 35°F., 40°F., 45°F. and 52°F. and also at room temperature.* All the fruits were kept in single layers in trays, each holding about thirty fruits. The temperature of the cold chambers did not show variation of more than 1°F. either way. The humidity was always recorded between 80 and 90 per cent. The condition of the fruit was examined at regular intervals during the storage period. The effect of temperature on green, turning and yellow fruits is described below.

GREEN AND TURNING FRUIT

At 35°F., the green fruits remained in a sound and green condition for eight weeks. The turning fruits kept in good condition for ten weeks. After these periods of storage, the fruits began to show signs of internal decomposition. As this internal breakdown advanced, the skin assumed a brown coloration and became soft, while the pulp gave a bitter taste. In the end all fruits decayed, the green after ten weeks and the turning after twelve weeks of storage.

At 40°F., the green fruits remained in the same condition for eight weeks and the turning kept well for twelve weeks. After ten weeks of storage, the green fruits began to turn slightly yellow. The fruits suffered in their juice content after six weeks of storage and the available juice after that period tasted insipid. Signs of internal fermentation were visible after ten weeks in the case of turning fruits. On prolonged storage the fruits decayed as at 35°F.

At 45°F., the green fruits began to change colour after two weeks of storage and became completely yellow in five weeks. The turning fruits were fully yellow after two weeks. Although both the green and turning fruits developed a good yellow colour, there was practically no juice available in the fruit. The pulp which was still quite firm had turned raggy. The fruits, however, did not show much tendency towards rotting. As the storage progressed, they got lighter and lighter until the pulp became dry. They could be kept in that condition for a long time afterwards.

---

*The experiments took place from 27th March to 30th April 1935, and the room temperature ranged from 77°F. to 90°F.

At 52°F., the green fruits began to change colour within two weeks and by the end of four weeks of storage had assumed an attractive orange colour but were without any juice. The turning fruits turned orange after a week only. These orange-coloured fruits, however, contained little juice in their pulp and the latter got dried up like that of fruits at 45°F., as the storage period advanced. The outward appearance remained unaffected and the fruits could be kept for a long time afterwards in that condition.

At room temperature, the green fruits kept as a control became wilted with a little change in the green colour. These fruits, however, failed to ripen properly.

YELLOW FRUITS

At 35°F., the ripe yellow fruits remained in a good condition retaining their original colour and full juice for ten weeks. After twelve weeks of storage the fruits started to give a strong fermenting odour and the juice was bitter in taste. The skin became dull and assumed a brown colour. Shortly afterwards, all the fruits showed rotting.

At 40°F., the fruits remained in a sound and juicy condition with the original colour for twelve weeks. After this, they suffered from gradual loss of juice to some extent. After sixteen weeks of storage, the fruits were not fit for use. The skin had turned dull and brown. The juice was bitter in taste and gave a strong alcoholic odour which indicated development of internal fermentation.

At 45°F., the fruits retained their colour and most of their juice for six weeks. On prolonged storage, the juice content gradually diminished and the pulp became almost dry after ten weeks. The fruits remained in the above condition for a long time afterwards (about four months) with little change in the outside appearance. The lightness of the fruits was evident when they were held in hand.

At 52°F., the fruits had their juice for the first four weeks only. After eight weeks of storage the pulp was practically dry. Otherwise their behaviour was similar to that of the fruits stored at 45°F.

At room temperature (77° to 90°F. on the average) the fruits kept as control first became wilted and then decayed completely within a fortnight after storage.

WASTAGE DURING STORAGE

The development of various moulds and fungi which cause rot to the Nagpur oranges require further investigation. It is, however, observed that during the early part of the storage period, the button (stem-end) turned dark brown with pitting of its skin, which spoiled the appearance of the fruit to a slight extent but did not damage the pulp. This development (Dark-button) was specially observed in the case of green and turning fruits.

At 35°F. and 40°F. and only in a few cases at 45°F. and 52°F., the fruits suffered from internal breakdown with the development of fermenting odour and bitter taste of the juice after varying periods of storage. The skin acquired a pale brown colour, became dull and collapsed in appearance. When the fruits in this condition were allowed to remain in chambers for a longer period, then only an attack by *Penicillium* moulds was observed.

Wastage due to a form of stem-rot was observed to an appreciable extent at the storage temperatures of 45°F. and 52°F. Usually the infection was noticed to penetrate through the 'Dark-button' and spread into the pulp by the central fibrous threads and at an advanced stage of development, the inside became almost black. Generally, at any rate, during the first stages of development of this type of stem-rot, the fruit appeared quite normal. It was difficult, therefore, to find out an affected fruit as there were no outward indications except perhaps that of a Dark-button.

Mycological examination* of the specimens of the fruits affected by Dark-button, 'internal breakdown' and stem-rot showed that :—

(*a*) No growth was obtained in cultures taken from the dark brown portion of the Dark-button, except in one case where *Diplodia* was isolated.

(*b*) A specimen of 'internal breakdown' which was badly rotting and gave out an acid smell showed the presence of yeast. From the discoloured skin of another specimen a culture bearing pycnidia of *Diplodia* was obtained.

(*c*) A specimen of stem-rot on cutting open showed the inside overgrown with black hyphae. There was no spore formation. In cultures, *Alternaria* was developed. Another specimen which was not very visibly diseased was found on cutting open to be black from inside. *Alternaria* was isolated from the blackened tissues.

(*d*) One of the specimens was fully rotten and dry. In cultures, *Colletotrichum* was developed.

(*e*) One specimen having stem-rot in the initial stages showed browning of the skin inside. It looked water-soaked. In cultures on incubation, pieces of the brown skin remained sterile.

Dark-button

The mode of development of Dark-button agreed with the description given in the report on Palestine oranges shipments [Empire Marketing Board, 1933] relating to a type of stem-rot which first appeared as a leathery brown discoloured area in the neighbourhood of the stem-end or button. An affected orange appeared quite normal since the fruit did not become specially soft or disintegrated. According to the report, however, this type of stem-end rot was caused by the fungus *Diplodia natalensis*. The Dark-button appeared to be similar to the Sore-eye or stem-end browning mentioned by Benton [1935].

---

* The authors are indebted to Mr. J. F. Dastur, Mycologist to Government, Central Provinces, Nagpur and Dr. B. N. Uppal, Plant Pathologist to Government, Bombay Presidency, Poona, for examining and identifying the specimens.

Internal breakdown

This type of decay was noticed by Young and Read [1931] in their experiments on Navel oranges and according to the description given by the authors, the skin acquired a pale yellow colour, became dull and mottled in appearance. They stated that it was the chief cause of wastage in one experiment in which the fruit was stored at 38° F. and came on after several weeks of storage. It did not occur in the same fruit stored at a temperature of 45° F. The 'internal breakdown' appeared to be a physiological trouble. Internal fermentation had set in as was evident from the presence of yeast which could be isolated.

Stem-rot

This type of rot appeared to be similar to the form of stem-rot, *Alternaria* disease, observed in Valencia oranges by Young and Read [1931] who described it as under :—

"This first makes its appearance after twelve weeks as a small brown area on the skin round the button, not extending into the fruit. These areas at a later period, about sixteen weeks, began to show definite infection with *Alternaria citri* Pierce, the mycelium of which penetrated the pith of the fruit and considerable area underneath the skin round the button. Later they developed typical black spores". They did not find this form of rot in their work on Navel oranges.

EFFECT OF KEEPING STALKS ON THE DEVELOPMENT OF DARK-BUTTON

A lot of oranges was obtained in which the stalks were not removed but half an inch of stalk was left on each fruit. These fruits behaved in the same manner as the other fruits without stalks. The development of Dark-button was not checked to any marked extent.

Chen [1934] found that soaking the fruit in five per cent borax solution at 32° to 40° C. for five minutes immediately after picking reduced *Penicillium* rot, but caused some injury to the stem-end. Nattrass [1935] secured the best control of decay by immersion in a cold saturated solution of borax for half a minute. The borax-treated fruit was softer than the control and although it became firm later, it did not compare favourably with control fruits. The results obtained by Winston [1935] indicate that immersion in borax immediately on arrival at the packing house is effective in retarding decay caused by stem-end rot. Benton [1935] found that the treatments in which borax was used gave appreciable and significant control of Sore-eye, and it was also prominent in controlling stem-end rot. Robson [1935] observed that green mould could be well controlled and blue mould less so, by immersing the fruit in a warm five per cent solution of borax and boric acid. A three per cent solution of sodium bicarbonate controlled blue mould but was less effective against the green.

Young and Read [1931] used solutions of borax, sodium bicarbonate, sodium sulphate and salicylic acid for washing oranges. Of these solutions only borax was found to have any effect in warding off attack by *Alternaria* in Valencias. They showed that wastage by *Alternaria* could be controlled to a large extent by treatment with borax followed with a coating of paraffin, the latter having a marked effect in preventing the attack of this fungus.

Friend and Bach [1932] observed that pre-storage treatments such as holding for ten days at 70°F. and wrapping in wax paper were highly beneficial whereas fungicidal washes were of no particular value. Guerrero [1927] found that dipping oranges in a 1 : 500 copper sulphate solution proved to be of no benefit.

PRESERVATIVE ACTION OF DIFFERENT TREATMENTS

Experiments were carried out to investigate the effect of different pre-storage treatments on Nagpur oranges. The fruits were given the following treatments :—

1. Washing with :—
   (*a*) Water.
   (*b*) Copper sulphate solution.
   (*c*) Potassium permanganate solution.
   (*d*) Borax solution (washed before despatch from Nagpur).
2. Besmearing with :—
   (*a*) Zinc oxide powder.
   (*b*) Magnesium oxide powder.
3. Coating with a thin film of paraffin wax.
4. Wilting at room temperature.

The observations on the storage of oranges treated in all the above ways showed no definite improvement in lengthening the period of storage as the development of Dark-button and the other forms of rotting were not prevented by different treatments given. As no rotting by *Penicillium* moulds was observed, the effect of the above treatments on these rots could not be studied.

The ripe fruits coated with paraffin wax remained in good condition for two months at 35°F. and 40°F., but after being taken out of the cold chambers, internal fermentation started on attaining the room temperature. The paraffin-coated fruits at 45°F. and 52°F. developed internal fermentation within a month of storage.

SUMMARY

The cold storage behaviour of Nagpur oranges of three different stages of maturity, *viz.*, green, turning and yellow has been studied. The results have shown that :—

1. Fully ripe yellow oranges could be kept in a good condition for three months at 40° F. without any appreciable wastage.
2. Green and turning fruits, although they changed to a good yellow colour at 45° F. and 52° F., lost their juice during storage.
3. Discoloration of the stem-end due to the development of Dark-button was specially marked in green and turning fruits in the beginning of the storage life.
4. At 35° F. and 40° F., fruits of the three stages of maturity developed 'internal breakdown' after varying periods of storage.
5. At 45° F. and 52° F., there was appreciable wastage due to *Alternaria* disease.
6. Pre-storage treatments such as washing with antiseptic solutions, etc., were of no particular value in lengthening the storage life of the fruits.

REFERENCES

Adam, D. B. (1923). *Jour. Dept. Agric. Victoria* **21**, 307-317.

Barker, J. (1935). "Food Industries Manual", London.

Benton, R. J. (1935). *Agric. Gaz. N. S. Wales* **46**, 389-93.

Chen, S. H. (1934). *Bull. Coll. Agric. Univ., Nanking* (China) **28** (n. s.)

Export of Oranges (1935). *Queensland Agric. Jour.* **44**, 81.

Friend, W. H. and Bach, W. J. (1932). *Texas Stn. Bull.* **446**.

Guerrero, J. (1927). *Guam Agric. Expt. Stn. Ann. Rept.*, 1927, p. 9.

Leonard E. R. (1936). *Low Temperature Research Station, Imperial College of Tropical Agriculture, Trinidad, Memoir No.* **2**.

Leonard, E. R. and Baker, R. E. D. (1934). *Trop. Agric.* **11**, 230.

McGuire L. P. (1929). *Trop. Agric.* **6**, 279-84.

Nattrass, R. M. (1935). *Cyprus Agric. Jour.* **30**, 84-7.

Palestine Orange Shipments (1933). *Empire Marketing Board, Report No.* **68**, July 1933.

Robson, G. (1935). *Food Manuf.* **10**, 159-61.

Rose, D. H. Wright, R. C., and Whiteman, T. M. (1933). *U. S. Dept. Agric Circular No.* **278**.

Tindale, G. B. (1927). *Jour. Agric. Victoria* **25**, 276.

Williams, W. J. (1935). *Hadar* **8**, 145-46.

Winston, J. R. (1935). *U. S. Dept. Agric. Tech. Bull.* **488**.

Young, W. J. and Read, P. M. (1931). *Proc. First Imp. Hort. Conf., Part III*, p. 50.

ONTOGENETIC DRIFTS IN THE PHYSIOLOGY AND CHEMISTRY OF TROPICAL FRUITS UNDER ORCHARD CONDITIONS

BY

B. N. SINGH, D.Sc.

P. V. V. SESHAGIRI, M.Sc.

AND

S. S. GUPTA, M.Sc.

Institute of Agricultural Research, Benares Hindu University, Benares

(Received for publication on 20th May 1936)

(With six text-figures)

THE PROBLEM : THE OUTLOOK AND MODE OF ATTACK

FROM the time the fruit is detached from the parent tree from which it draws its nourishment, the individual fruit shows progressive ageing, heading fast to senescence, culminating in death and decay. The rate at which the metabolic drift proceeds within the separated fruit goes a long way to determine its subsequent life, and it is governed by the conditions inside the fruit and the environment in which it is placed. Thus a detailed investigation into the drift of metabolism and the chemical changes occurring within the fruit in natural conditions should be the necessary prelude to any work undertaken to prolong the life and keeping quality of the fruit. Likewise it is important to know the pre-storage conditions of the fruit likely to influence the storage life.

Considerable progress has been made in the preservation and storage of fruits both in England and America, and the literature is well covered in a series of interesting articles by workers in these regions. The complexity of the problem, however, becomes more acute in the tropics where on account of the extreme conditions of intense illumination and high temperature, the metabolic and chemical changes proceed at a rapid rate to the detriment and wastage of the fruits.

In the present investigations two lines of attack have been followed : firstly, investigations of the changes on the tree and secondly, investigations of the changes in store, of fruits gathered at different dates. The former study includes investigations of changes taking place throughout the development of the fruit, as the so-called ripening period is continuous with, and inseparable from, the growing period. In the latter aspect is included a study of the chemical changes occurring in fruits after gathering in storage, realising that a detailed knowledge of the chemical changes with the progress of senescence is likely to contribute something towards the efforts to prevent wastage in store owing to physiological diseases and rots.

It is aimed to trace the relationship between chemical constituents and the keeping quality of the fruits, as such a study may be helpful in the production of fruits suitable for any given purpose, by varying their composition by such treatments of the tree as manuring, pruning and ringing. The former aspect of the study of changes of the fruit on the tree is dealt with in the present paper.

Mango and guava, being widely distributed and used all over India, are selected as materials for investigation. The respiratory index and quotient of the developing fruits at successive stages of the ontogeny, the weekly dry-matter production, the total acidity and the *p*H value, the osmotic pressure of the fruit-sap and chemical analysis of the material with respect to reducing sugars, sucrose, starch, total nitrogen, acidity, etc., are determined, and the nature of the relationship between the diverse physiological and chemical changes attendant upon growth and development of fruits under natural environments of the orchards worked out.

METHODS AND MATERIALS

The investigations are mainly confined to pure strains of *Mangifera indica* (vars. Langra and Krishnabhog) and *Psidium guava* (var. Benares local) growing at the experimental orchards attached to this Research Station. With the appearance of the flowers labelling is done, and soon after setting has taken place the still developing flowers are removed.

Picking is done a day previous to experimentation. The fruits are sterilised with mercuric chloride solution (1 : 1000), washed with distilled water and allowed to rest overnight in the laboratory at a temperature of 30°C. in order to unify the previous history of the experimental material.

Growth is measured on the basis of dry-matter production. Fruits are plucked every week, and after recording the morphological and other characteristics they are dried in a steam oven at a temperature of 100°C. The growth rate is calculated by the formula $R/100 = \log_e W_2 - \log_e W_1$, where W_1 and W_2 are the weights of two successive weeks, R the growth-rate and 'e' the base of the natural logarithm.

The measurement of carbon dioxide output is made both by continuous current method, using baryta solution as an absorbent [Inamdar and Singh, 1927], and by the Singh and Mathur's modified Haldane's gas analysis apparatus [1936]. The respiratory intensity of the fruit is expressed in terms of grams of carbon dioxide per hour per kilogram fresh weight, as well as per kilogram dry weight of the material. The changes in the water content from period to period varying quite disproportionately to the weight of the fruits, necessitated our studying the respiratory drifts in terms of both their fresh and dry weights. In the case of mango the respiratory quotients of the different parts of the fruit have been studied with a view to determine their relative intensities of respiration.

For the determination of the osmotic pressure the flesh of the mango fruit is cut off and the sap expressed from it. The freezing point of the sap is found by the Beckman's apparatus (cryoscopic technique). After making due correction for under-cooling, the osmotic pressure is calculated from the corrected freezing point by the formula $P = 12{\cdot}06\Delta - 0{\cdot}021\Delta^2$.

To proceed with the estimation of sugars, about 100 grm. of the fresh fruits are cut up finely and are soaked in 200 c.c. of ninety per cent alcohol for twelve hours after adding a small quantity of calcium carbonate to neutralise the acids present. The alcohol is filtered off and the material is extracted with hot alcohol in a Soxhlet's apparatus for six hours. The cold and hot extracts are mixed and the alcohol is distilled off under reduced pressure. The remaining liquid is dissolved in distilled water and the solution is detanned by basic lead acetate and deleaded by the addition of saturated sodium phosphate solution. The solution is filtered and made up to volume. The reducing power of this solution is determined volumetrically using Lane and Eynon's method [1923], in which methylene blue is used as an internal indicator. The total sugars are estimated as reducing sugars after inversion of 100 c.c. of the above solution by ten per cent citric acid and neutralising the acid by an alkali. The values for sucrose are obtained by finding the difference between total sugars and reducing sugars and expressed in terms of invert sugar.

For the estimation of starch the alcohol-insoluble residue is digested for six hours with 100 c.c. of 2·27 per cent hydrochloric acid solution. The extract is filtered, evaporated on a water-bath to a few c.c. and the residual liquid is dissolved in distilled water. Basic lead acetate solution is added to detan the solution and the excess of lead is precipitated by the addition of sodium phosphate solution. The liquid is filtered and made up to volume. The reducing sugars present are estimated by the above method.

A portion of the well-ground paste of the fresh material is mixed with distilled water and the suspension is filtered through a cintered glass funnel under reduced pressure. A known quantity of centinormal sodium carbonate solution usually in excess, is added and back-titrated against centinormal hydrochloric acid. The total acidity is expressed in terms of c.c.'s of alkali used to neutralise the acids present in one gram of the fresh material.

The *p*H of the sap is determined by the electrometric method using the quinhydrone electrode.

The total carbohydrates in guavas are estimated by digesting the material with 2·27 per cent hydrochloric acid and estimating the reducing sugars as given bove.

Nitrogen is estimated by the well-known Kjeldahl's method [Skinner, 1930] modified to include organic and inorganic nitrogen.

EXPERIMENTAL RESULTS AND DISCUSSION

Physiological and chemical changes in the developing fruits

Absolute growth and growth rate.—When the growth magnitudes are plotted in terms of dry matter at successive stages of the life-cycle of the mangoes and the guavas, the time-weight curves show the usual sigmoid type (Table I). The essential features of the growth curves of plants on the one hand and animals on the other, as discussed elsewhere [Singh, 1932], are characteristically noted in the case of the fruits as well. Fruits thus constitute independent physiological units having their characteristic growth curves very much like those of individual plants or animals.

TABLE I

Absolute growth of fruits during the ontogeny

	Dry weight in grms. per fruit														
Age in weeks	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Langra	0·5	1·20	2·08	5·60	9·5	13·8	16·4	18·2	19·5	20·6	21·5	20·8	20·8	...	...
Krishnabhog . . .	...	0·25	0·15	0·32	0·63	0·85	2·14	3·91	7·66	12·84	15·67	18·40	21·5	21·4	20·5
Guava . . .	1·81	2·18	2·40	3·12	5·05	9·25	14·20	16·20	18·16	19·20	19·76	20·24	20·4	20·4	...

The relative growth rate (Fig. 1) increases rapidly during the early periods of the life-cycle and later decreases with age till the end in Langra mangoes and guavas. In the Krishnabhog variety, however, the rate is high in young stages and gradually declines with age with a slight rise in the sixth week. The high rate of growth during the early stages may be connected with an increased activity of the rapidly multiplying cells in the formation of new tissues necessitating a rapid supply of nourishment from the tree.

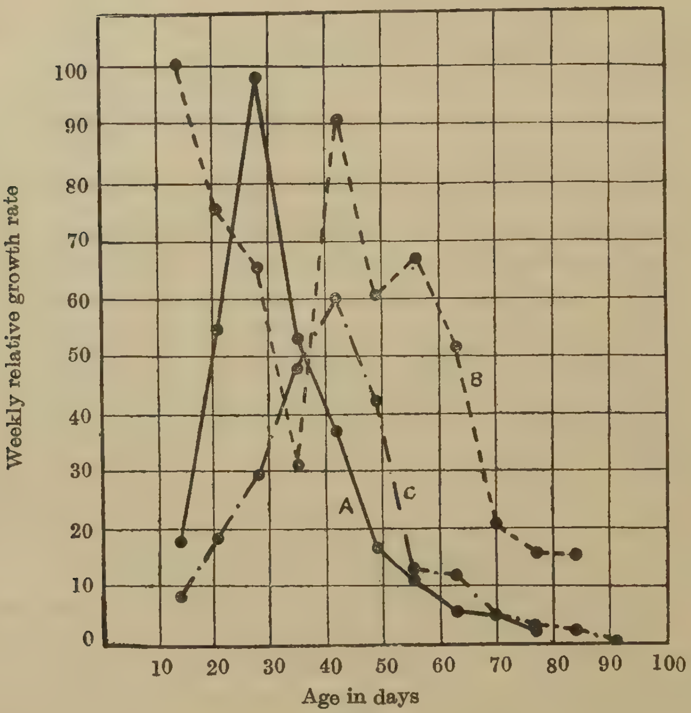


Fig. 1

Relative growth rate of Langra mangoes (A), Krishnabhog mangoes (B), Guavas (C) in successive weeks.

Langra ; 17·85 ; 55·01 ; 99·04 ; 52·85 ; 37·34 ; 17·26 ; 10·41 ; 6·90 5·49 ; 2·40.

Krishnabhog ; 101·25 ; 75·994 ; 65·713 ; 30·781 ; 91·932 ; 60·324 ; 67·25 51·652 ; 19·915 ; 15·995 ; 15·675.

Guava ; 8·49 ; 19·03 ; 29·56 ; 48·35 ; 61·08 ; 42·39 ; 12·28 ; 12·04 ; 5·55 ; 2·83 ; 2·39 ; 0·80.

It is interesting to note that the growth rate runs parallel to the nitrogen values (Fig. 4), the increasing and decreasing growth activities following closely the increasing and decreasing nitrogen contents throughout the life-cycle of the fruits. In view of the fact that the nitrogen is directly or indirectly related to protein-nuclein content of the protoplasm, its presence may, to a certain extent, be associated with the amount of protoplasm in the fruit tissue. Hence high nitrogen values are likely to be associated with greater protoplasmic content and consequently higher rate of growth and *vice versa.*

Respiratory intensity.—The output of carbon dioxide in mangoes (Fig. 2) calculated on the basis of fresh as well as dry weights, gradually increases till the twenty-eighth day in the case of Langra and forty-second day in the case of Krishnabhog. During this period the activity of the meristematic cells as well as the growing cotyledons accounts for the characteristic rise in respiration values. The later decline, when the stony endocarp is fully developed, may be attributed to a lag in the diffusion of carbon dioxide through the hard endocarp enveloping the growing cotyledons.

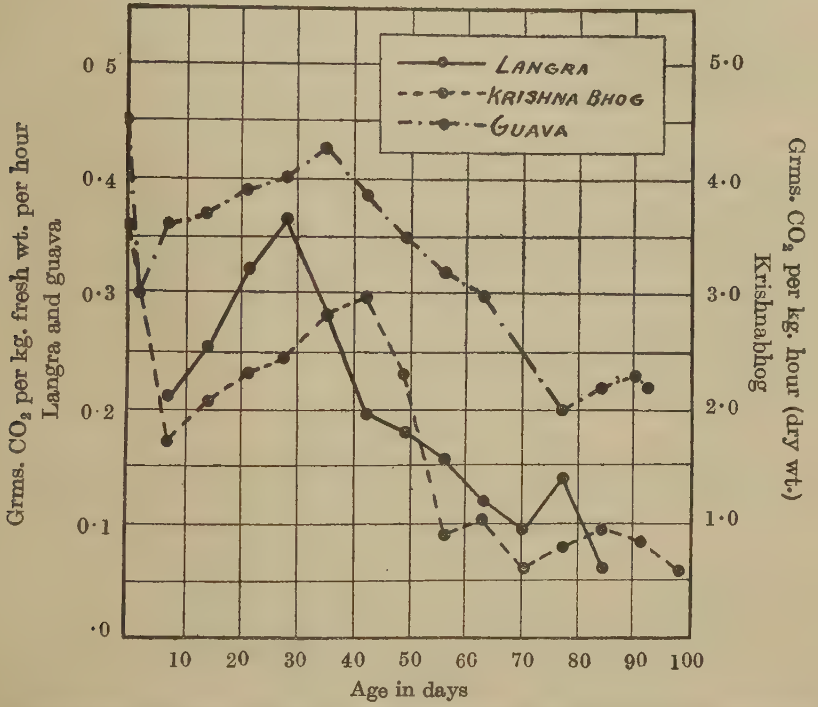


Fig. 2. Respiratory indices of Langra mangoes, Krishnabhog mangoes and guavas at successive weeks in the ontogeny.

Langra : 0·2095 ; 0·2521 ; 0·3210 ; 0·3658 ; 0·2854 ; 0·1935 ; 0·1802 ; 0·155 ; 0·1220 ; 0·0950 ; 0·1350 ; 0·0600 grams of carbon dioxide per kilogram fresh weight per hour.

Krishnabhog : 3·35 ; 1·71 ; 2·05 ; 2·30 ; 2·45 ; 2·80 ; 2·97 ; 2·29 ; 0·90 ; 1·04 ; 0·61 ; 0·82 ; 0·95 ; 0·87 ; 0·60 ; grams of CO_2 output per kilogram dry weight per hour.

Guava : 0·450 ; 0·298 ; 0·360 ; 0·371 ; 0·392 ; 0·405 ; 0·423 ; 0·386 ; 0-350 ; 0·320 ; 0·301 ; 0·252 ; 0·200 ; 0·220 ; 0·225 , 0·251 ; 0·240 gramsof CO_2 output per kilogram fresh weight per hour.

The nut and cotyledons in the case of Langra variety are carefully separated from the fleshy mesocarp, and their rates of respiration are determined. The carbon dioxide output of the cotyledons is found to increase up to the twenty-eighth day (Table II) like that observed in the case of entire fruit, to be subsequently followed by a continuous decline. The magnitude of respiration is characteristically higher than that for the entire fruit. The nut with the cotyledons enclosed therein exhibits a considerable fall in the respiratory intensity when compared to that of the cotyledons alone, indicating thereby that the endocarp acts as a physical barrier to the free outward diffusion of carbon dioxide. On the thirty-fifth day the respiration of the nut is lesser than that of the cotyledons alone, yet it remains above the respiratory level of the fruit as a whole. This fact suggests that the mesocarp is also partially responsible for the obstruction of gaseous exchange. With advancing age, the thickness of the endocarp increases and the diffusion of carbon dioxide is greatly prevented leading to a decline in the respiratory index of the nut much below that of the respiratory level of the entire fruit. At still later stages the respiratory curve of the nut touches the zero line when the maximum thickness of the endocarp is reached (Table II). The fruit attains its maximum size at this stage, after which the colour changes from greenish yellow to golden yellow. During this stage the respiratory intensity shows a characteristic rise and fall between the seventieth and eighty-fourth days in Langra, seventieth and ninety-eighth days in Krishnabhog, similar to that observed by Blackman and Parija [1928] and Kidd and West [1930] on apples.

TABLE II

Respiratory index and respiratory quotient changes in langra mangoes

Age in weeks	Respiratory index			Respiratory quotient		
	Entire fruit	Cotyledons	Nut	Entire fruit	Cotyledons	Nut
1 . .	0·2095	..	..	1·010	..	..
2 . .	0·2521	0·2540	..	1·050	1·010	..
3 . .	0·3210	0·3380	..	0·850	1·020	..
4 . .	0·3658	0·4290	..	0·850	1·010	..
5 . .	0·2854	0·3948	0·3294	0·780	0·989	1·002
6 . .	0·1935	0·2670	0·1850	0·889	0·979	1·012
7 . .	0·1802	0·2200	0·1720	0·990	1·001	1·021
8 . .	0·1550	0·2080	0·1200	1·090	0·995	0·997
9 . .	0·1220	0·1850	0·0981	1·110	1·025	0·994
10 . .	0·0950	0·1375	0·0749	1·127	1·082	0·902
11 . .	0·1350	0·1292	0·0125	1·180	1·201	0·851
12 . .	0·0600	0·9810	0·0108	1·200	1·312	0·804

The respiratory course of the Krishnabhog variety too is similar to the one observed for Langra variety although slight differences in the location of the maximal humps are noted probably owing to their racial difference. In both these varieties the fruits mature by the end of the tenth-eleventh week, after which yellowing appears with the result that they may fall down irrespective of their chronological age. To avoid complications on this score the mature fruits of the same chronological age and developmental stage are removed in lots from the orchard and stored in the laboratory. In the senescent stage, therefore, our observations on the metabolic drifts and chemical composition have been recorded mainly upon the detached fruits stored in the laboratory.

The intensity of respiration in the guavas (Fig. 2) is very high in the flowering stage, to be soon followed by a marked decline as the fruits set. Guavas do not exhibit any remarkable feature in their respiratory drifts during the early stages as observed in mangoes, apparently on account of the difference in structure, in the absence of nut and cotyledons of the size of those in mangoes. A senescent rise in respiration is noted like the one observed in mangoes. The characteristic rise and fall in senescent mangoes and guavas are analogous to those observed in a previous study from these Laboratories in the case of oranges [Singh, 1930] wherein the drifting increase in the carbon dioxide output during the senescent stage has been ascribed to a progressive rate of hydrolysis to produce greater respirable substrate, consequent to an enhanced rate of carbon dioxide output, brought about by a lowering in the organisational resistance of the fruits.

Respiratory quotient.—This in the case of the Langra mangoes rises slightly during the first fifteen days of development, followed by a decline, steep at first and steady later on, till the fruits are thirty-five days old (Table II). This low respiratory quotient may be due to the greater production and storage of acids, a fact which has been experimentally shown to be true on the basis of our observations recorded for the titratable acids (Fig. 5). As the acids break down towards the senescent period, the quotient reaches a higher value than unity.

The respiratory quotient of the cotyledons from their formative stage up to the fifty-sixth day is very nearly unity, afterwards increasing to 1·3 as the age advances. In the case of nut, however, the unit values in the first sixty-three days are later followed by low values of the respiratory quotient.

Water-content and osmotic pressure.—The water-content of the Langra variety varies from a maximum of eighty-five to a minimum of seventy per cent while in the Krishnabhog variety (Table III) it varies between ninety and sixty-four per cent according to the stage of development. With decreased water-content there occurs a rise in the osmotic values (Table III). This relationship is found to hold towards the senescent stage when an enormous increase in the osmotic pressure is accompanied by a decrease in the hydration factor, and seems to have a pronounced influence on the senescent drift of these fruits. It would thus appear that if the water-content of the fruits be maintained at a steady level the fruits may keep longer and prolong their senescent stage considerably, a fact which is confirmed from certain direct observations on the storage life of the fruits in relation to humidity [Singh, 1932]. This hydration factor is not only important in bringing about the changes in the storage life of the fruits, but also is an important controlling variable in the shedding of fruits [Singh and Ambegaokar, 1931] during their early stage.

The fall in hydration factor towards the end of the ontogeny of these fruits is accompanied by a continuous rise in the osmotic pressure which exhibits a decline after eighty-four days. It may be that the presence of high concentration of sucrose (Fig. 3) in the flesh of mangoes is responsible for this high osmotic pressure.

TABLE III

Water-content and osmotic pressure in Krishnabhog mangoes

—	Age in weeks												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Water-content .	90·0	90·12	87·55	85·88	81·05	71·50	75·62	75·24	74·81	70·30	66·48	65·0	64·8
Osmotic pressure	...	...	...	...	7·05	...	17·90	...	25·00	...	52·15	43·5	...

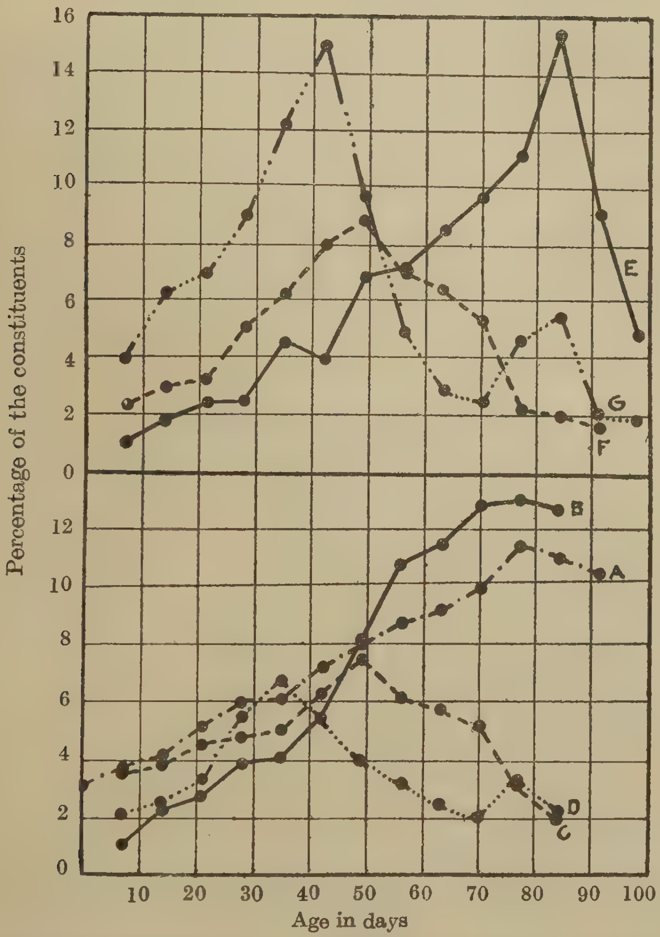


Fig 3. Total carbohydrates in guava (A); Sucrose (B), Starch (C), Glucose (D) in Langra mangoes. Sucrose (E), Starch (F) and Glucose (G) in Krishnabhog mangoes.

Total carbohydrates in guava (A): 3·100; 3·70; 4·125; 5·10; 6·05; 6·10; 7·20; 8·00; 8·80; 9·20; 10·03; 11·5; 11·23; 10·50.

Sucrose in Langra (B): 1·01; 2·21; 2·80; 3·90; 4·10; 5·50; 8·10; 10·80; 11·50; 12·90; 13·12; 12·81.

Starch in Langra (C): 3·50; 3·80; 4·50; 4·80; 5·00; 6·30; 7·50; 6·20; 5·80; 5·23; 3·10; 2·05.

Glucose in Langra (D): 2·00; 2·50; 3·30; 5·50; 6·80; 5·50; 4·02; 3·19; 2·50; 1·98; 3·15; 2·09.

Sucrose in Krishnabhog (E): 1·092; 1·937; 2·74; 2·53; 4·62; 4·00; 6·90; 7·21; 8·56; 9·80; 11·20; 15·49; 9·18; 5·005.

Starch in Krishnabhog (F): 2·38; 2·95; 3·30; 5·08; 6·28; 8·01; 8·90; 7·005; 6·46; 5·50; 2·30; 2·05; 1·75.

Glucose in Krishnabhog (G): 4·12; 6·258; 6·95; 9·02; 12·18; 14·95; 9·73; 5·00; 3·036; 2·63; 4·85; 5·63; 2·205; 2·03.

All the constituents are expressed as percentages in terms of fresh weight of the material.

Chemical changes in the developing fruits

March of carbohydrates.—In Langra mangoes the reducing sugars (Fig. 3 D) increase till the thirty-fifth day and later decline till the seventieth day, with a corresponding rise and fall in the respiratory index (Figs. 2, 3). A rise till the seventy-seventh day and a decrease in the glucose content in the end, run parallel to the respiratory activity of the fruits. This similarity in the nature of the curves for both the respiratory intensity and percentage of reducing sugars indicates the possibility of hexoses acting as the material substrate for respiration, such that an increase or decrease in the one is followed by a corresponding response in the other. In Krishnabhog mangoes the reducing sugars (Fig. 3 G) exhibit two maxima similar to those in the other variety although differences are to be noted in the location of the maximal humps.

Sucrose is found to accumulate continuously throughout the life-cycle of both the Langra and Krishnabhog varieties (Fig. 3 B, E) as a result of translocation from the tree. The slight decline in the percentage of sucrose during the later stage is noted, probably connected in some way with the operation of starvation factor at this stage.

The starch contents of both the Krishnabhog and Langra varieties (Fig. 3 F, C) indicate a rise till about the first forty-nine days of the life-history, to be subsequently followed by a drop in the values. The decrease in the percentage of starch towards the later stages is associated with an increase in the sucrose content of the fruits, suggesting the probable hydrolysis of starch into sucrose. The accumulation of sugars especially sucrose at the cost of starch during the senescent stage gives a clue to the fact that the greater sucrose content of the fruits may, to a certain extent, depend upon the availability of starch towards the pre-senescent stage.

Guavas, although resembling mangoes with respect to their general march of carbohydrates, differ from them inasmuch as an increase in the total carbohydrate content continues till a period of seventy-seven days beyond which a decline is noticed (Fig. 3 A). It is thus evident that in the ripening of fruits there is a rapid loss of starch and sucrose (Fig. 3) which may be due to their consumption directly or indirectly in the process of respiration. With the depletion of the store of respirable substrate the life of the fruit is also cut short. *Ipso facto* it would follow that the storage life of the fruit may be extended if the rate of respiration is lowered by storing the fruits under conditions favourable to a lower rate of depletion of the food materials contained in them.

Nitrogen changes in the ontogeny.—The nitrogen content of Langra mangoes and guavas (Fig. 4) increases gradually till the twenty-eighth and thirty-fifth days, respectively, with subsequent decline during the later developmental stages, whereas in Krishnabhog mangoes nitrogen content increases till the twenty-first day and then depicts a downward trend with many fluctuations. There is a rapid loss towards the later stages, probably connected with the physiological breakdown of tissues during the senescent stage or related to the process of slow protein oxidation and accumulation of poisonous substances as suggested by Archbold [1925]. Further, nitrogen being a measure of the protoplasm indicates that its increase in the earlier stages may be due to the building up of the new tissues.

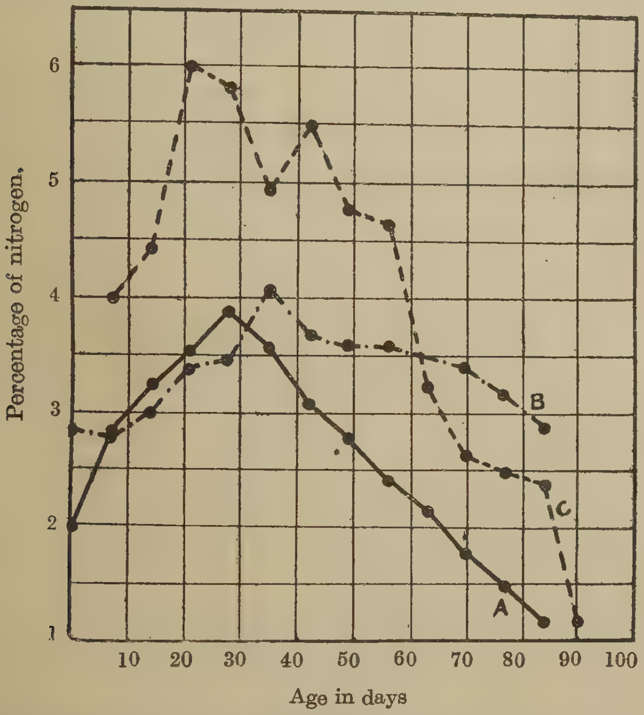


Fig. 4. Percentage of nitrogen expressed in terms of fresh weight in Langra mangoes (A), Krishnabhog mangoes (C) and Guavas (B).

Langra (A) : 0·20 ; 0·283 ; 0·325 ; 0·361 ; 0·389 ; 0·360 ; 0·310 ; 0·243 ; 0·215 ; 0·181 ; 0·151 ; 0·12.

Krishnabhog (C) : 0·400 ; 0·445 ; 0·600 ; 0·585 ; 0·495 ; 0·550 ; 0·480 ; 0·465 ; 0·325 ; 0·265 ; 0·250 ; 0·24 ; 0·12.

Guava (B) : 0·285 ; 0·280 ; 0·308 ; 0·339 ; 0·353 ; 0·420 ; 0·367 ; 0·363 ; 0·359 ; 0·350 ; 0·349 ; 0·343 ; 0·32 ; 0·292.

The identity in the course of nitrogen drift and growth rate (Fig. 1) emphasises a close relationship between the two. Up to the twenty-eighth day there is an increase in growth rate in Langra mangoes with increasing nitrogen content while with the decline in nitrogen content there is to be noted a simultaneous fall in the growth rate. As was previously observed the variations in the nitrogen content agree fairly closely with similar changes in the respiratory rate. The fruits with low nitrogen content being thus usually characterised by low respiration rate, should be better suited for storage.

The carbohydrate/nitrogen ratio.—The carbohydrate/nitrogen ratio in these fruits exhibits a continuous ascending phase from the early stage of fruit development, attaining a maximum value when the fruits are completely mature (Table IV). A decline in the growth rate is usually accompanied by a rise in the carbohydrate/nitrogen ratio. In young fruits the ratio is low, being apparently due to the low carbohydrate contents accompanied by high values of nitrogen. With increase in the carbohydrates towards maturity the ratio once again rises. A high carbohydrate/nitrogen ratio therefore is characteristic of fully developed fruits.

TABLE IV

Carbohydrate/nitrogen ratio during the ontogeny

——	Age in weeks												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Krishnabhog mangoes.	13·03	23·70	20·60	27·10	44·22	45·81	51·32	45·40	57·20	70·49	86·20	95·0	111·5
Guavas . .	13·21	13·38	15·04	16·29	14·52	19·59	22·05	24·54	26·28	29·50	33·05	35·10	35·9

Total acidity and p*H.*—The acid content of the Krishnabhog mangoes rises slowly in the early stages up to the sixty-third day in the life-cycle and falls rapidly towards the end (Fig. 5 i). There is gradual storage of acids possibly due to an incomplete combustion of the respirable materials caused by the hindrance in the proper exchange of gases through the mesocarp and epicarp. By the sixty-third day the highest acid content is reached in Krishnabhog while in the Langra variety acidity is highest by the forty-ninth day. In view of this if the fruits are to be used in the preparation of pickles and acid powders, where high acidity is of primary importance, it may be worth-while to remove Langra variety from the tree when they are forty-nine to fifty-six days, while Krishnabhog mangoes may be picked at a stage between sixty-three and seventy days of their maturity,

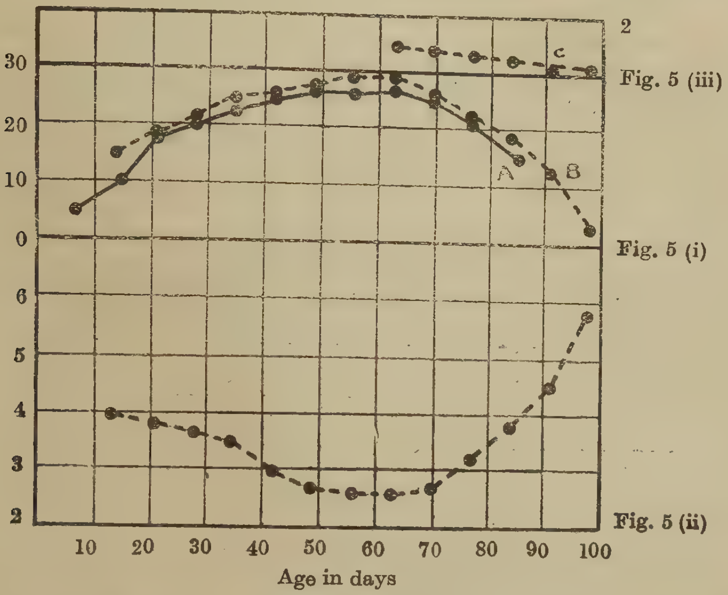


Fig. 5 (i). The titratable acidity of the Langra mangoes (A) and Krishnabhog mangoes (B)—

Langra (A) : 5·20 ; 10·20 ; 18·0 ; 20·3 ; 22·5 ; 25·1 ; 26·8 ; 26·2 ; 25·8 ; 25·4 ; 21·0 ; 15·0.

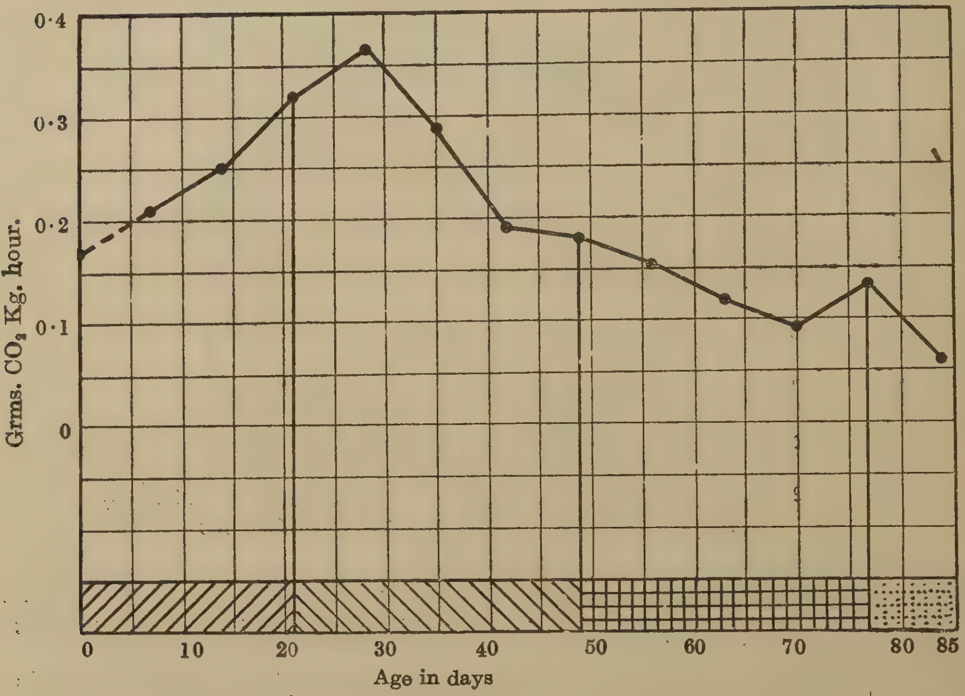
Krishnabhog (B) : 15·20 ; 18·95 ; 21·50 ; 25·05 ; 26·15 ; 27·5 ; 29·0 ; 28·9 ; 26·05 ; 22·5 ; 18·8 ; 13·20 ; 2·80.

(ii) Weekly values of *p*H in *Krishnabhog* mangoes 4·0 ; 3·8 ; 3·65 ; 3·50 ; 3·0 ; 2·7 ; 2·65 ; 2·62 ; 2·80 ; 3·20 ; 3·80 ; 4·50 ; 5·83.

(iii) Curve (C) represents the log of the values of acidity of the Krishnabhog mangoes.

The curve for total acidity is found to run in a direction opposite to that of *p*H (Fig. 5 ii) ; this is so since the value of *p*H is the logarithm of the reciprocal of hydrogen-ion concentration, which in its turn is proportional to the total amount of acids present. The acid content falls rapidly in senescent Krishnabhog mangoes. The logarithmic curve obtained for the decline of acidity is a straight line, proving thereby that the fall in the acidity following the logarithmic rule, proceeds at a definite rate (Fig. 5 iii). During the periods of acid accumulation starch is also being stored, while the depletion of the former is simultaneously accompanied by the hydrolysis of the latter to sugars. It may thus be inferred that the acidity of the fruits may in some way govern the storage and hydrolysis of starch.

Having discussed the physiological and chemical changes that may be taking place within the fruits, we would justifiably divert our attention to an analysis of the life-history of the individual mango fruit in the above light, stressing upon the changes characteristic of each stage. The life-history of the mango fruit may thus be segregated into following four more or less well-definable stages (Fig. 6).



JUVENILE	ADOLESCENT	CLIMACTERIC	SENESCENT
Rapid growth, high respiratory index. Carbohydrates, nitrogen, acids increasing. C/N low.	Growth, respiration, water contents declining, starch steady, acids, nitrogen at maximum. C/N increasing.	Respiration minimum. Starch, nitrogen, acids declining. Sucrose, flavour, odour highest. Osmotic pressure maximum.	Respiration rise and fall. Starch, acid glucose, nitrogen, sucrose, declining.

Fig. 6. A gist of the physiological, chemical and physical changes at the four developmental stages in the ontogeny of fruits in general.

Juvenile.—This ends in about twenty-one days after fertilisation when the fruit is about the size of a lemon. It is characterised by a stage of rapid cellular growth, high respiratory intensity, high growth rate, maximum water-content, and steady increase in carbohydrates, nitrogen, and acid content. The carbohydrate/nitrogen ratio is low. The epicarp of the fruit is green in colour, firmly attached to it.

Adolescent.—This stage runs between twenty-one and forty-nine days. This is identified by increase in size to a maximum, developing aroma and flavour, medium rate of respiration, declining growth rate, increasing osmotic pressure, and decreasing water-content. The glucose percentage rapidly falls, sucrose fastly increases, starch maintains a high level, the acids and nitrogen contents show fairly high values with increasing carbohydrate/nitrogen ratio. The fruit assumes now a dark green colour.

Climacteric stage.—It is a critical stage in the ontogeny of the fruit extending between forty-nine and seventy-seven days when the respiration is minimum and starch is steadily hydrolysed to sugars with a fall in acid content. Sucrose exhibits a maximum percentage at this stage. A fall in nitrogen content is noticed accompanied by a high carbohydrate/nitrogen ratio. The climacteric marks a beginning of senescence accompanied by a change of colour from dark green to yellowish green.

Senescent stage.—It extends from the seventy-seventh day onward, depending upon the variety, characterised by the rise in the rate of respiration followed by a decline. The growth of fruits is not marked, osmotic pressure increases, and water-content attains a low level. Glucose content increases and decreases with corresponding rise and fall in respiratory intensity, while sucrose and starch exhibit an appreciable decline. The acidity of the fruit decreases. A decline in nitrogen is noticed, with a rise in the carbohydrate/nitrogen ratio. Yellowing of the epicarp of the fruit is marked. The fruit becomes soft, the internal tissue disintegrates, and finally death ensues with decay and decomposition of the material constituents.

SUMMARY AND CONCLUSIONS

In the present paper an attempt has been made to study the natural drift of metabolic changes in developing fruits at successive stages of the ontogeny.

Krishnabhog and Langra mangoes and Benares local guavas are selected for experimentation and certain aspects of the physiology and chemistry of these fruits studied.

The growth rate increases during the earlier stages followed by a continuous decline towards maturity. The respiratory index of the entire fruit exhibits two maxima, one during the early stage and the other during the senescent stage. The respiratory drift runs more or less parallel to the percentage of reducing sugars at all stages of development.

The declining respiratory activity at later stages in mangoes has been experimentally shown to be due to hindrance in the gaseous exchange through the hard stony endocarp and the fleshy mesocarp of the mango fruits. The cotyledons are the most actively respiring organs when compared to the nut and the fruit as a whole. The nut during the earlier stages has higher respiratory index as compared to that of the entire fruit, but as the endocarp thickens the values fall down to reach the zero line towards the close of the life-cycle.

The respiratory quotient exhibits two maxima—the first during the early juvenile stage followed by a steady decline during the adolescent stage and the second during the climacteric and senescent stages. An inverse relationship is indicated between the acid contents of the fruits and the respiratory quotient, an increase in the former leading to a decrease in the latter, and *vice versa.*

A decrease in the hydration factor results in an increase in the osmotic values of the sap. This has been shown to be due to the increasing sucrose content.

The reducing sugars increase both during early and later stages of growth according to the variations in respiratory intensity of the fruit. The sucrose exhibits a continuous rise. The rise in the later period is also explained on the basis of starch hydrolysis. The starch percentage shows a single maximal hump during the beginning of the climacteric phase.

The nitrogen content fluctuates from period to period in a direction similar to that of the respiratory index. A high nitrogen content is thus accompanied by a higher rate of respiration, and *vice versa.* There is a positive correlation between nitrogen content and growth rate of the fruits. The carbohydrate/nitrogen ratio is low during the younger stages, gradually rising to a higher value with advance in age.

The *p*H values run in a direction opposite to those of total acidity attaining a maximum towards the initiation of the climacteric stage. With the accumulation of acids starch also increases, the depletion of the former rising parallel to the hydrolysis of the latter in the senescent stage.

On the basis of the physiological and chemical changes the life-cycle of the fruit in general has been segregated into four more or less well-defined stages:—

Juvenile, extending between seven and twenty-one days.
Adolescent, extending between twenty-one and forty-nine days.
Climacteric, extending between forty-nine and seventy-seven days.
Senescent, extending from seventy-seven days onwards.

Each stage in the fruit development is characterised by certain specific physiological and chemical changes depending, to a larger extent, upon the racial differentiation of the fruits.

REFERENCES

Archbold, H. K. (1925). *Ann. Bot.* **39**.

Blackman, F. F. and Parija, P. (1928). *Roy. Soc. Proc. B.* **103**.

Inamdar, R. S. and Singh, B. N. (1927). *Jour. Ind. Bot. Soc.* **6**, Nos. 3 and 4.

Kidd, F. and West, C. (1930). *Roy. Soc. Proc. B.* **106**.

Lane, J. H. and Eynon, L. (1923). *Jour. Soc. Chem. Ind.* pp. 32 T.

Singh, B. N. (1930). *Proc. Ind. Sci. Cong. Bot. Sec., Allahabad.*

———— (1932). "The basis of ageing, rejuvenation and death." Malaviya Commemoration Volume. Indian Press Ltd., Allahabad.

Singh, B. N. and Ambegaokar, K. V. (1931). *Proc. Ind. Sci. Cong. Bot. Sec., Nagpur.*

Singh, B. N. and Mathur, P. B. (1936). *New Phytol.* **35**, No. 5.

Skinner, W. W. (1930). "The Official and Tentative Methods of Analysis: Association of Official Agricultural Chemists, Washington," 3rd edition, p. 593.

STATISTICAL NOTES FOR AGRICULTURAL WORKERS

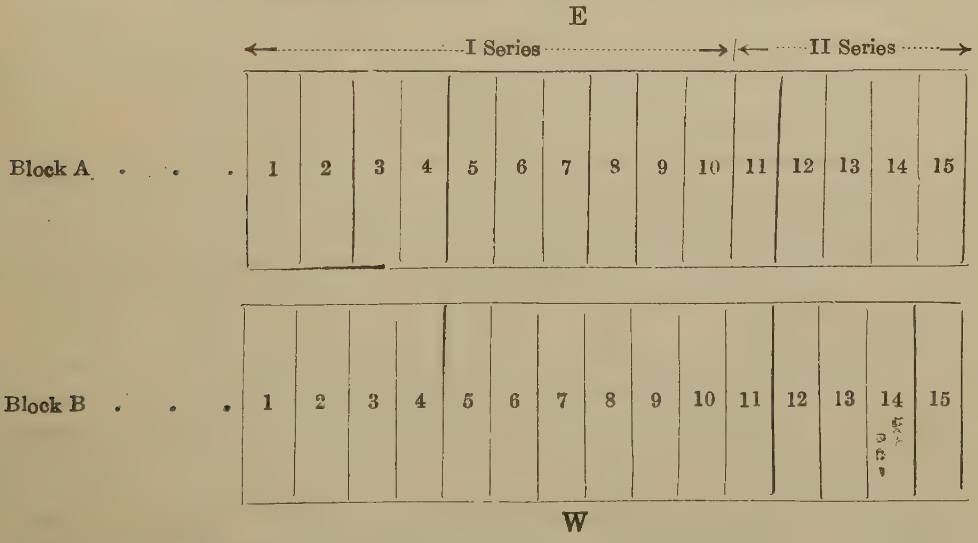
No. 22. A STATISTICAL STUDY OF THE YIELDS OF THE PERMANENT MANURIALS, PUSA

BY
T. V. G. MENON, B.A., B.Sc. (Agri.), Assoc. I. A. R. I.
Imperial Institute of Agricultural Research, Pusa
AND
S. S. BOSE, M.Sc.
Statistical Laboratory, Calcutta

(Received for publication on 29th October 1936)

Introduction

The Permanent Manurial and Rotation Experiments at Pusa were laid out in 1908 in two blocks A and B each with fifteen plots; each plot was one quarter of an acre in size, lying east to west as shown in the diagram below.


E
I Series
II Series
Block A
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
Block B
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
W

These experiments were started with a view to study the conditions which determine soil-fertility in a sub-tropical climate, and in particular the manner in which soil-fertility is affected by the application of important manures including green manure, by rotation of crops with special reference to the part played by legumes in a rotation, by cultivation, and by fallows [Shearer, 1908].

The scheme was as follows :—

Series I.—The object of this series was to determine the specific effect on soil-fertility of the more important organic and chemical manures applied alone and in various combinations to a two years' four-course rotation. Thus all the plots of this series in blocks A and B had in the first year maize in the *Kharif* season followed by *arhar* as the *Rabi* crop and in the second year maize was followed by oats. The four-course rotation maize-*arhar*-maize-oats was thus complete in every two years and this was repeated for eleven cycles. We thus have twenty-two crops of maize and only eleven crops of *arhar* and oats during the period 1908-09 to 1929-30.

The manurial dressings were applied to maize every year, so that the *Rabi* crop received only the residual effect of the manure if any. The treatments were as follows :—

(1) No manure.

(2) Farmyard manure to supply 10 lbs. nitrogen.

(3) Farmyard manure to supply 20 lbs. nitrogen.

(4) Farmyard manure to supply 30 lbs. nitrogen.

(5) Rape cake to supply 20 lbs. nitrogen.

(6) Ammonium sulphate to supply 20 lbs. nitrogen.

(7) Potassium sulphate to supply potash as in farmyard manure No. 3.

(8) Superphosphate to supply phosphoric acid as in farmyard manure No. 3.

(9) Potassium sulphate to supply potash as in farmyard manure No. 3.
Superphosphate to supply phosphoric acid as in farmyard manure No. 3.

(10) Ammonium sulphate to supply nitrogen as in farmyard manure No. 3.
Potassium sulphate to supply potash as in farmyard manure No. 3.
Superphosphate to supply phosphoric acid as in farmyard manure No. 3.

Series II.—" This series was designed to determine how far soil-fertility was affected by growing in a rotation leguminous crop (1) removed from the land, (2) returned to the land in the shape of green manure. It is complementary to series I, inasmuch as the results obtained will give an indication of how far legumes can replace manures in a rotation. All crops of each rotation were grown in the same year." [Shearer, 1908.]

The rotational scheme and soil-treatments are shown below :—

	Kharif	*Rabi*	*Kharif*	*Rabi*	Remarks
(11)	Maize .	Barley .	Maize .	Oats .	No manure.
(12)	,, . .	,, .	Sann-hemp	,, .	Green manure on cereal rotation.
(13)	,, . .	*Arhar* .	Maize .	,, .	Residual value of deep-rooted pulse.
(14)	,, . .	,, .	,, .	Oats and peas	Residual values of two legumes, one deep and one shallow-rooted.
(15)	,, . .	,, .	Sann-hemp	Oats .	Residual value of a pulse crop *plus* a green manure.

[Harrison, 1929] has discussed the treatment differences by a comparison of the mean yields. A summary of his conclusions is given below.

(*i*) *The effect of organic manures.*—Farmyard manure has increased the croppings in cereals, but the extra increase is not marked with amount above 20 lbs. nitrogen per acre. The increase in maize with rape-cake is almost equal to that given by farmyard manure at 30 lbs. nitrogen per acre, but with oats, which gets the residual effect only, its influence is negligible. Rape-cake is inferior to farmyard manure and its residual effect is small. The different dressings of farmyard manure have given the same grain production in *arhar*. The effect of rape-cake is small.

(*ii*) *Mineral manures.*—Nitrogen and potash have both depressed the yields of the cereals, the depression being greatest in the case of potash. Of the manurial constituents, nitrogen, potash and phosphoric acid the latter is the only one which gives positive reaction, but the combination of all the three gave the best results. In the case of cereals phosphoric acid has done well. The addition of potash to superphosphate has had no advantage. The complete manure is superior to superphosphate or farmyard manure alone. In oats the effect of the mixture is only slightly better than that of superphosphate.

(*iii*) *Legumes.*—In maize the benefit due to the inclusion of legumes in rotation is positive, but the increase is not appreciable. A deep-rooted legume in cereal rotation in oats has shown a slight increase. In *arhar* shallow-rooted legume has resulted in a slight decrease. There is a definite depletion of yield of *arhar* under the influence of green manure even in conjunction with superphosphate as in the case of a second leguminous crop. It would seem as if the use of green manure in conjunction with a leguminous crop of the type of *arhar* were deleterious. But the green manure has greatly benefited the cereal crops. The legume in rotation has given only a comparatively small increased benefit. Green manure and superphosphate in combination has done well.

It is of interest to compare these conclusions with those drawn in this paper (pages 211-14) as a result of statistical analysis.

METHOD OF ANALYSIS

In this paper the yields of grain of maize, *arhar* and oats in the two series, A and B, have been taken separately and analysed by Fisher's analysis of variance. It has been possible to find (*a*) the respective influence of the two blocks A and B; (*b*) the effect of the different manurial dressings; (*c*) the effect of the seasons; (*d*) the differential response of the treatments with respect to the seasons; (*e*) the effect of treatments on the blocks with respect to the crops.

It will be seen that in plots 12 and 15, maize was grown in alternate years, these therefore have been omitted from the analysis of maize yields. Plots 11 and 12 did not grow *arhar*, so that there were thirteen treatments under *arhar*. Oats were however grown in alternate years over all the fifteen plots of each block. The analysis of variance for the yields of the three crops are shown in Tables I, II and III.

TABLE I

Analysis of variance of yields of maize (in lbs. per acre)

	Degrees of freedom	Sum of squares	Mean square	Ratio
Block	1	1551267	1551267**	71·695
Treatment	12	18808957	1567413**	72·441
Regression	1	14333808	14333808**	662·467
Deviation	20	20703585	1035179**	47·843
Years	21	35037393	1668447**	77·111
Treatment × years	252	12617164	50068*	2·314
Residual	285	6166632	21637	
	571	74181413		

TABLE II

Analysis of variance of yields of arhar

	Degrees of freedom	Sum of squares	Mean square	Ratio
Block	1	18368	18368	0·264
Treatment	12	4561474	380123**	5·456
Regression	1	1117831	1117831**	16·045
Deviation	9	5098182	566465**	8·131
Years	10	6216013	621601**	8·922
Treatment × years	120	3706061	30884	0·443
Residual	142	9893039	69669	
	285	24394955		

TABLE III

Analysis of variance of yields of oats

	Degrees of freedom	Sum of squares	Mean square	Ratio
Block . . .	1	461965	461964**	7·853
Treatment . .	14	10438504	745607**	12·675
Regression . .	1	6739	6739	0·115
Deviation . .	9	5587754	620862**	10·554
Years . . .	10	5594493	559449**	9·510
Treatment × years .	140	2374536	16961	0·288
Residual . .	164	9647368	58825	
	329	28516865		

We notice in these tables that—

(1) The treatment differences are significant;

(2) The seasonal differences are significant and that a steady deterioration is noticeable in maize and *arhar*, although other types of fluctuations are also present.

(3) Except for *arhar*, the two blocks have given different mean yields of maize and oats.

(4) For maize, the relative efficiencies of the manurial treatments showed significant variations from season to season, or in other words, there was a differential response of the treatments to seasons.

These points may now be studied quantitatively with reference to mean yields.

SYSTEMATIC SOIL DIFFERENCE

The experiment was laid out in two replications on two adjacent parallel strips of land, the eastern block or block A and the western block or block B. The mean yields of these two blocks for all treatments over all the years are shown in Table IV.

TABLE IV

Mean yield of blocks in lbs.

	Block A	Block B	Mean	Standard error
Maize in lbs. . . .	771·39	667·23	719·31	8·70
Percentage	107·24	92·76	100·00	1·29
Arhar in lbs. . . .	851·79	835·76	843·78	22·07
Percentage . . .	100·95	99·05	100·00	2·61
Oats in lbs.	673·55	598·72	636·14	18·88
Percentage	105·88	94·12	100·00	2·97

For all the crops block A yielded more than block B, but the difference was statistically significant for maize and oats and not for *arhar*.

SEASONAL FLUCTUATION

The tables of analysis of variance definitely indicate that for all crops, the mean yields vary considerably from year to year. The annual means of the three crops together with percentages are shown in Table V.

TABLE V

Mean yield of maize, arhar *and oats in lbs.*

	Maize		*Arhar*		Oats	
	Mean	Per cent	Mean	Per cent	Mean	Per cent
1908-09	1312·00	182·40	..	..	458·97	72·15
1909-10	484·00	67·29	955·81	113·28	..	..
1910-11	1249·04	173·64	..	..	838·13	131·75
1911-12	906·88	126·08	1126·54	133·51	..	..
1912-13	981·69	136·48	..	..	759·23	119·35
1913-14	771·85	107·30	957·65	113·50	..	..
1914-15	675·31	93·88	..	..	614·93	96·67
1915-16	894·50	124·36	878·00	104·06	..	..
1916-17	854·77	118·83	..	..	675·53	106·19
1917-18	889·04	123·60	581·58	68·93	..	..
1918-19	715·19	99·43	..	..	507·37	79·76
1919-20	632·08	87·87	850·00	100·78	..	..
1920-21	468·35	65·11	..	..	573·20	90·11
1921-22	746·54	103·79	769·88	91·24	..	..
1922-23	618·92	86·04	..	..	474·33	74·56
1923-24	624·77	86·86	644·42	76·37	..	..
1924-25	429·42	59·70	..	..	734·00	115·38
1925-26	222·32	30·91	742·19	87·96	..	..
1926-27	540·54	75·15	..	..	545·20	85·70
1927-28	522·42	72·63	841·58	99·74	..	..
1928-29	489·23	68·01	..	..	816·60	128·37
1929-30	718·15	99·84	933·88	110·68	..	..
Mean yield	719·31	100·00	843·78	100·00	636·14	100·00
Standard error	28·85	4·01	51·76	6·13	44·28	6·96

Thus the yield of maize varied from 1312 lbs. in 1908-09 to only 222lbs. in 1925-26. The fluctuations of *arhar* and oats yield were not so marked, but still varied between ± 30 per cent of the mean yield. These differences must be attributed to weather factors, *e.g.*, rainfall, sunshine, temperature, humidity, etc., together with such variations as changes in methods of cultivation, tillage and other operations from year to year on one hand and to steady changes and other fluctuations in the soil due to continuous cropping and similar treatments on the other.

The tables of analysis of variance indicate that a steady deterioration accounts for an appreciable part of the seasonal changes for maize and *arhar* but not for oats. The deviations from linear regression are also significant, so that in addition to the linear annual diminution there are other types of changes of a non-linear type. The amount of deterioration for individual treatments has been discussed in a separate paper.

EFFECT OF TREATMENTS

The scheme of treatments from (1) to (10) can be divided into four equivalent groups with reference to nitrogen, phosphoric acid and potash contents of the different doses of farmyard manure, while treatments (11) to (15) form a new one. This is shown in Table VI.

TABLE VI

Scheme of treatment equivalents

1 1 No manure	2 2 F. Y. M.	3 3 F. Y. M.	4 4 F. Y. M.	5 Green manure
	(1st dose) = 10 lbs. N	(2nd dose) = 20 lbs. N (5) Rape cake with N equivalent to 2nd dose F. Y. M. (6) $(NH_4)_2SO_4$ with N equivalent to 2nd dose of F. Y. M.	(3rd dose) = 30 lbs. N (7) K_2SO_4 with K equivalent to 3rd dose of F. Y. M. (8) Superphos with P equivalent to 3rd dose of F. Y. M. (9) K_2SO_4 + Superphos with K and P equivalent to 3rd dose of F. Y. M. (10) K_2SO_4 + Superphos + $(NH_4)_2SO_4$ with K, P and N equivalent to 3rd dose of F. Y. M.	

The mean yields of maize is shown in Table VII and according to this scheme in Table VIII.

TABLE VII

Mean yield of maize, arhar *and oats in lbs. under different treatments*

	Maize		*Arhar*		Oats	
	Mean	Per cent	Mean	Per cent	Mean	Per cent
1. Control .	574·84	79·92	947·86	112·33	566·86	89·11
2. F. Y. M. (= 10 lbs. N) .	753·61	104·77	1006·68	119·31	709·09	111·47
3. F. Y. M. (= 20 lbs. N) .	896·85	124·70	1068·86	126·68	822·55	129·30
4. F. Y. M. (= 30 lbs. N) .	991·91	137·90	972·95	115·31	907·77	142·70
5. Rape cake (= 20 lbs. N)	861·66	119·79	898·91	106·53	616·05	96·84
6. Amon. sulph (=20 lbs. N).	547·45	76·11	880·82	104·39	449·68	70·69
7. Pot. sulph .	485·18	67·45	769·36	91·18	425·18	66·84
8. Superphos .	845·16	117·50	830·14	98·38	822·64	129·32
9. Pot. sulph + Super .	803·61	111·72	786·91	93·26	800·14	125·78
10. Amon.sulph + Pot. sulph +Superphos.	975·82	135·66	771·36	91·42	867·77	136·41
11. Legume crop . .	472·95	65·75	..	..	428·82	67·41
12. Green manure . .	..	..	..	..	635·45	99·89
13. Deep-rooted pulse .	543·66	75·58	721·77	85·54	456·55	71·77
14. Deep + shallow root .	598·23	83·17	649·14	76·93	347·91	54·69
15. Green manure + pulse crop . .	..	..	664·32	78·73	685·36	107·74
Mean yield .	719·31	100·00	843·78	100·00	636·14	100·00
Standard error.	22·17	3·08	56·27	6·67	51·71	8·13

Table VIII

Mean yield of maize in lbs.

Control	F. Y. M. (1)	F. Y. M. (2)	F. Y. M. (3)	Green manure and legumes
(1) 574·84	(2) 753·61	(3) 896·95 (5) 861·66 (6) 547·45	(4) 991·91 (7) 485·18 (8) 845·16 (9) 803·61 (10) 975·82	(11) 472·95 (13) 543·66 (14) 598·23

Standard error = 22·17

Table IX

Mean yield of arhar *in lbs.*

Control	F. Y. M. (1)	F. Y. M. (2)	F. Y. M. (3)	Green manure and legumes
(1) 947·86	(2) 1006·68	(3) 1068·86 (5) 898·91 (6) 880·82	(4) 972·95 (7) 769·36 (8) 830·14 (9) 786·91 (10) 771·36	(13) 721·77 (14) 649·14 (15) 664·32

Standard error = 56·27

Table X

Mean yield of oats in lbs.

Control	F. Y. M. (1)	F. Y. M. (2)	F. Y. M. (3)	Green manure and legumes
(1) 566·86	(2) 709·09	(3) 822·55 (5) 616·05 (6) 449·68	(4) 907·77 (7) 425·18 (8) 822·64 (9) 800·14 (10) 867·77	(11) 428·82 (12) 635·45 (13) 456·55 (14) 347·91 (15) 685·36

Standard error = 51·71

Three doses of farmyard manure gave increasing yields of maize ; the increase due to first dose is 179 lbs. per acre ; the increase due to second dose over first is 143 lbs. per acre ; the increase due to third over second is 95 lbs. per acre. It appears probable that a decreasing return is setting in, although the actual increases are all significant. In group (2), rape-cake and ammonium sulphate have all 20 lbs. of nitrogen in common. Rape-cake gives the same yield as farmyard manure (2) but the difference is not so marked as that between farmyard manure (2) and ammonium sulphate (6). In fact, with ammonium sulphate the yield is lower than the control. In group 3, the complete equivalent manure (10) gives a slightly lower yield than farmyard manure (3). The phosphate equivalent (8) comes next in this group, but its yield per acre is 147 lbs. less than farmyard manure (3). Potassium sulphate by itself or in combination with superphos has depressed the yield very considerably.

The 5th group treatments as a rule have not shown any response, except treatment (14) with two legumes that show a small improvement (of the order of four per cent) over control.

We thus notice that for maize in Pusa, farmyard manure is by far the best, the complete manure is the next approach ; of the three constituents, nitrogen, phosphorus and potash, phosphorus seems to be the greatest benefactor while potash exhibits a depressing effect. The green manure and legume treatments are not very effective.

RESIDUAL EFFECT OF MANURE

Arhar.—Arhar did not receive the direct effect of the manures ; it followed maize and consequently received only the residual effect of the dressings. The mean yields are shown in Tables VII and IX.

The farmyard manure plots did not show any residual effect as these yields were identical with those of the untreated plots. But the plots treated with other fertilisers were all below the level of the control plots in yield. Treatment (10) which contained nitrogen, phosphorus and potash in the same proportion as in farmyard manure (3) yielded 176 lbs. to the acre less than the control plots. The plots with legumes and green manures were worse.

Oats.—Oats like *arhar* received the benefit of the residual effect of manures. The yields are shown in Tables VII and X.

The plots with farmyard manure gave appreciable increases over the control with regard to oat yields. The complete artificial manure (10) and phosphate (8) also showed this improvement. Potash in combination with phosphate (9) showed no added benefit and by itself (7) was worse. Plots treated with ammonium sulphate (6) yielded much less than even control. The green-manured areas were without any residual effect ; in fact, some of them, particularly the double legume (treatment 14) gave poorer yields compared to the untreated plots.

Discussion

It will be seen from Tables VII-X that farmyard manure with thirty lbs. of nitrogen gave the best results for all the crops. For maize and oats the lower doses of farmyard manure have always given significantly lower yields but for *arhar* the residual effect of the three doses have been identical. The complete chemical fertiliser (No. 10) with the same amount of nitrogen, phosphorus and potash as the third dose of farmyard manure gave the same yield as the farmyard manure for maize and oats, but for *arhar* the yield was worse than the yield of the untreated plot. Rape-cake proved effective for maize as well as for the subsequent *arhar* crop but not for oats. Superphosphate showed good response in the case of maize and oats but not for *arhar*. This failure in the case of *arhar* for all superphosphate combinations may be due to the growth of wilt (*Fusarium vasinfectum* Atk.) which superphosphate has been found to encourage [McRae and Shaw, 1933]. Potash has been uniformly without any response.

The green-manure treatments or the legumes did not exhibit any improvement for any of the three crops and sometimes gave smaller yields than the control.

Finally it should be mentioned that the lay-out of the experiment was unsatisfactory from two points of view of—

(1) Want of randomisation.

(2) Inadequate replication.

The first defect leads to a biased estimate of error and the second to a loss of precision. The analysis however has been based on the assumption of randomness in the lay-out and the discussion has been made in the light of the accuracy attained, however inadequate.

Summary

1. The yields of the maize, *arhar* and oats in the Permanent Manurial and Rotation Experiment at Pusa, from 1908-09 to 1929-30 have been analysed to find the effects of the blocks, seasons, treatments and the interaction between these factors with respect to the yield. Although the plots were not properly randomised or replicated, R. A. Fisher's method of analysis of variance has been used on the assumption that the condition of randomness was approximately satisfied. All results obtained here are subject to this assumption.

2. The analysis shows that the blocks gave distinctly different yields in the case of maize and oats and nearly so in *arhar*.

3. The influence of seasonal fluctuations on the yields of the three crops was significant.

4. Maize was manured every year, while the succeeding *Rabi* crops, *arhar* and oats in alternate years, received only the residual effect.

In the case of maize and oats farmyard manure (= 30 lbs. nitrogen) per acre and the complete manure (ammonium sulphate *plus* potassium sulphate *plus* superphosphate equivalent to farmyard manure giving 20 lbs. nitrogen) gave the best results. But in *arhar*, farmyard manure (= 20 lbs. nitrogen) gave the highest yields.

5. The effect of rape-cake on maize and *arhar* was more or less similar. The residual effect of rape-cake was not so good as that of farmyard manure.

6. Superphosphate comes next in rank to rape-cake in its effect on maize. In the case of *arhar* it has not acted beneficially and the effect is worse even than the control. This is explained from the presence of wilt (*Fusarium vasinfectum* Atk.) which superphosphate has been shown [McRae and Shaw, 1933] to encourage.

7. Potash by itself did not benefit the crops in any way.

8. Ammonium sulphate also did not show appreciable results except in *arhar* for which there was a slight response.

9. Green manure was practically without any effect.

ACKNOWLEDGMENTS

The authors' best thanks are due to Dr. W. McRae, late Director, Imperial Institute of Agricultural Research, Pusa, for the interest evinced in the subject, for facilities afforded in the preparation of the paper and his guidance from time to time. For permission to work in the Statistical Laboratory and his guidance in the statistical analysis the authors are greatly indebted to Professor P. C. Mahalanobis of Calcutta. Thanks are also due to Mr. Anil Chandra Nag, M.Sc., of the Statistical Laboratory for help rendered in the reduction and analysis of these data.

REFERENCES

Harrison (1929). *Proceedings of the Board of Agriculture in India*, App. I.

McRae, W. and Shaw, F. J. F. (1933). *Sci. Monograph No. 7 of the Imperial Council of Agricultural Research.*

Shearer (1908). *Proceedings of the Board of Agriculture in India*, App. B, p. 37.

STATISTICAL NOTES FOR AGRICULTURAL WORKERS

NO. 23. A STATISTICAL STUDY OF THE DETERIORATION OF YIELDS OF THE PERMANENT MANURIALS, PUSA

BY

S. S. BOSE, M.Sc.
Statistical Laboratory, Presidency College, Calcutta

AND

T. V. G. MENON, B.A., B.Sc. (Agri.), Assoc. I.A.R.I.
Imperial Institute of Agriculture, Pusa

(Received for publication on 29th October 1936)

The Royal Commission on Indian Agriculture [1928] referred to soil deterioration in the following questions :—

> " Can you give instances of soil known to you which, within your recollection, have suffered marked deterioration ? "

This enquiry excluded the alkali salt tracts of the Punjab or the regions rendered unfit for cultivation as a result of soil-erosion. The real question was " whether long-cultivated agricultural land is today suffering a growing diminution in its capacity to yield crops, as a consequence of the removal, year by year, in the form of produce, of more of these substances essential to the growth and development of crops than is replaced by nature and by the practice of the cultivator " (page 75). On this issue, the Commission was informed that in Bombay, Bengal and Burma there was no evidence of any decline in the yield of staple crops, while in Madras, the United Provinces and the Punjab, the tendency was towards a slight increase in outturn. The Government of Bihar and Orissa considered the available statistics insufficient to justify any definite conclusion being drawn from them. In the opinion of the Agricultural Adviser to the Government of India, most of the area under cultivation in India has been under cultivation for hundreds of years and had reached its state of maximum impoverishment many years ago.

The Commission on the basis of the evidence at its disposal stated," While paucity of records of crop outturn throughout India over any long period of time makes the matter impossible of exact proof, we are of opinion that the strong presumption is that an overwhelming proportion of agricultural lands of India long ago reached the condition to which experimental data point. A balance has been established and no further deterioration is likely to take place under existing conditions of cultivation." (page 76).

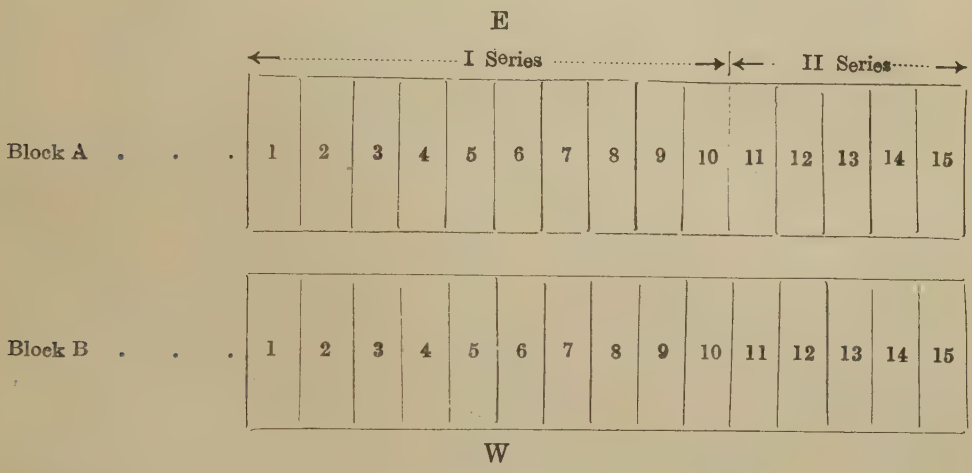
It may be added that data for average yield of a particular crop, say rice or wheat, over a long period for the whole of India or of a province do not supply the necessary information as it is well known that increased pressure of population upon the land often forces the cultivator to till lands with inferior soil or additional areas with limited resources as regards supplies of manures, seeds, etc.; and the cultivation of such marginal lands naturally results in decreasing crop outturn per acre. For a scientific investigation, it is necessary to note either at the same station or at a number of stations distributed at random over a given countryside, records of crop outturn over a long period of time, say thirty years or more, and then subject the data to statistical analysis. A notable example of this type of work is to be seen in R. A. Fisher's " An Examination of the Yield of Dressed Grain from Broadbalk " [1921] in which is given a survey of the results of a statistical examination of the yield of the plots of Broadbalk wheat field with continuous cropping for sixty-seven years (1852—1918).

Fisher distinguished three types of variation in the wheat yield: (1) annual fluctuations, (2) steady diminution due to deterioration of the soil, and (3) slow changes other than steady diminution, and estimated the changes in yield due to each of these factors. He also calculated the probabilities of large diminutions occurring by chance owing to particular later seasons happening to be on the average less favourable than the earlier ones.

In the present paper which covered only twenty-two years such detailed analysis is not possible. An attempt has therefore been made to study the question of only steady deterioration. It must be remembered, as Fisher has pointed out, that the deteriotation must not be assumed to be mathematically linear. On most plots it was probably more rapid in the earlier years than it was later (page 109, footnote).

MATERIAL AND METHOD

The present analysis is based on the yield data of a Permanent Manurial Test conducted at Pusa during 1908—1929 obtained through the courtesy of Mr. T. V. G. Menon of Pusa. The experiment was laid out in two blocks, A and B, each with fifteen plots of $\frac{1}{4}$ acre each. There were fifteen treatments in duplicate as shown below :—



Plan of the experiment

The cropping scheme was mostly a four-course, two-year rotation—Maize-*arhar*-maize-oats—and the manurial dressings were applied direct to the maize crop every year ; the oat and *arhar* thus received only the residual effect of the treatments.

Plots 12 and 15 did not grow maize in alternate years and were therefore omitted from studies on maize ; for the same reason, plots 11 and 12 were omitted for *arhar* ; oat was grown over all the fifteen plots and all these have been included in the analysis.

For determining the amount of annual diminution a linear equation: $y = a + b\,(t - \bar{t})$ was fitted to the series of yield-data of each individual plot. Here t represents the year, a the mean yield of the plot over the given period, and b the annual change. A negative value of b indicates a diminution and a positive value an improvement in the yield.

The values of a and b, the mean and the rate of change, are shown in Tables I—III for two blocks of each crop. The figures are in lbs. per acre ; thus in Table I, we note that the untreated plot in block A gave a mean yield of 658·27 lbs. of maize per acre and that the yield was found to diminish at the rate of 22·28 lbs. per acre per year.

TABLE I

Amount of steady soil-deterioration of maize yield in lbs.

Block A						Block B					
Treatments	Mean yield (lbs. per acre)	Deterioration per acre		Regression coefficient		Treatments	Mean yield (lbs. per acre)	Deterioration per acre		Regression coefficient	
		(lbs. acre)	(Per cent)	5 per cent	1 per cent			(lbs. acre)	(Per cent)	5 per cent	1 per cent
1	2	3	4	5	6	7	8	9	10	11	12
1. Control	658·27	—22·28	—3·38	17·04	21·64	1. Control	491·41	—22·59	—4·60	13·53	17·18
2. F. Y. M. (= 10 lbs. N)	815·45	—18·54	—2·27	15·68	19·92	2. F. Y. M. (= 10 lbs. N)	691·77	—21·86	—3·16	21·31	27·07
3. F. Y. M. (= 20 lbs. N)	977·64	—18·36	—1·88	19·55	24·82	3. F. Y. M. (= 20 lbs. N)	816·27	—21·41	—2·62	18·64	23·67
4. F. Y. M. (= 30 lbs. N)	1088·77	—9·50	—0·87	17·05	21·65	4. F. Y. M. (= 30 lbs. N)	895·05	—11·17	—1·25	20·16	25·60
5. Rape-cake (= 20 lbs. N)	938·59	—23·86	—3·54	21·80	27·68	5. Rape-cake (= 20 lbs. N)	784·73	—30·72	—3·91	20·73	26·33
6. Amon. sulph (= 20 lbs. N)	586·91	—29·81	—4·93	18·88	23·97	6. Amon. sulph (= 20 lbs. N)	508·00	—24·73	—4·87	24·83	31·53
7. Pot. sulph	547·00	—23·90	—4·37	23·19	29·45	7. Pot. sulph	423·36	—20·46	—4·83	23·74	30·15
8. Superphos	910·86	—27·31	—3·00	20·03	25·43	8. Superphos	779·45	—25·74	—3·30	20·42	25·93
9. Pot. sulph + Super	861·32	—23·18	—2·69	19·75	25·08	9. Pot. sulph + Super	745·91	—31·14	—4·17	20·84	26·46
10. Amon. sulph + Pot. sulph + Superphos	976·55	—32·78	—3·36	21·35	27·11	10. Amon. sulph + Pot. sulph + superphos.	975·09	—34·35	—3·52	23·82	30·25
11. Legume crop	464·27	—39·44	—8·50	18·31	23·26	11. Legume crop	481·64	—38·05	—7·90	22·50	28·58
12. Green manure	...	...	...	...	...	12. Green manure	...	...	...	...	...
13. Deep-rooted pulse	579·05	—39·87	—6·89	17·64	22·40	13. Deep-rooted pulse	508·27	—31·64	—6·23	24·22	30·76
14. Deep + shallow root	623·36	—38·32	—6·15	16·01	27·33	14. Deep + shallow root	573·09	—31·94	—5·57	22·31	28·97
15. Green manure + pulse crop	...	...	...	...	...	15. Green manure + pulse crop.	...	...	...	...	...

TABLE II

Amount of steady soil-deterioration of arhar *yield in lbs.*

Block A						Block B					
Treatments	Mean yield (lbs. per acre)	Deterioration per acre		Regression coefficient		Treatments	Mean yield (lbs. per acre)	Deterioration per acre		Regression coefficient	
		(lbs. per acre)	(Per cent)	5 per cent	1 per cent			(lbs. per acre)	(Per cent)	5 per cent	1 per cent
1	2	3	4	5	6	7	8	9	10	11	12
1. Control	958·45	—43·53	—4·54	55·79	68·09	1. Control.	937·27	—36·29	—3·87	51·17	62·44
2. F. Y. M. (= 10 lbs. N)	974·91	—22·91	—2·35	52·71	64·32	2. F. Y. M. (= 10 lbs. N)	1038·45	—17·97	—1·73	42·32	51·65
3. F. Y. M. (= 20 lbs. N)	1026·91	+6·72	+0·65	42·51	51·88	3. F. Y. M. (= 20 lbs. N)	1110·82	—17·15	—1·54	45·70	55·77
4. F. Y. M. (= 30 lbs. N)	1041·27	—8·42	—0·81	44·39	54·18	4. F. Y. M. (= 30 lbs. N)	904·64	—41·44	—4·58	42·86	52·31
5. Rape-cake (=20 lbs. N)	928·64	—6·08	—0·65	47·20	57·60	5. Rape-cake (= 20 lbs. N)	869·18	—29·64	—3·41	32·23	39·33
6. Amon. sulph (= 20 lbs. N)	914·73	—12·52	—1·37	54·67	66·72	6. Amon. sulph (=20 lbs. N)	846·91	—7·42	—0·88	31·54	38·50
7. Pot. sulph	783·91	—11·24	—1·43	49·63	60·57	7. Pot. sulph	754·82	—32·35	—4·29	30·04	36·67
8. Superphos	822·00	—15·86	—1·93	54·42	66·42	8. Superphos	838·27	—63·20	—7·54	22·15	27·03
9. Pot. sulph + super	743·73	—20·07	—2·70	58·56	71·47	9. Pot. sulph + super	830·09	—24·21	—2·92	67·31	82·15
10. Amon. sulph + pot. sulph + superphos	741·27	—33·56	—4·52	63·70	77·74	10. Amon. sulph + pot. sulph + superphos	801·45	—21·34	—2·66	59·92	65·35
11. Legume crop	...	...	...	...	...	11. Legume crop	...	...	...	...	...
12. Green manure	...	...	...	...	...	12. Green manure	...	...	...	...	...
13. Deep-rooted pulse	758·36	—13·96	—1·84	58·79	63·97	13. Deep-rooted pulse	685·18	—16·72	—2·44	39·56	48·28
14. Deep + shallow root.	667·73	—9·56	—1·43	53·41	65·18	14. Deep + shallow root	630·55	—13·16	—2·09	40·59	49·53
15. Green manure + pulse crop	711·36	+4·01	+0·56	47·94	58·50	15. Green manure + pulse crop.	617·27	—10·70	—1·73	40·17	49·02

TABLE III

Amount of steady soil-deterioration of oats yield in lbs.

Block A						Block B					
Treatments	Mean yield (lbs. per acre)	Deterioration per acre		Regression coefficient		Treatments	Mean yield (lbs. per acre)	Deterioration per acre		Regression coefficient	
		(lbs. per acre)	(Per cent)	5 per cent	1 per cent			(lbs. per acre)	(Per cent)	5 per cent	1 per cent
1	2	3	4	5	6	7	8	9	10	11	12
1. Control	648·55	—46·05	—7·10	42·57	51·95	1. Control	485·18	+1·99	+0·41	38·91	47·49
2. F. Y. M. (= 10 lbs. N)	763·27	—11·25	—1·47	36·60	44·01	2. F. Y. M. (= 10 lbs. N)	654·91	+10·48	+1·60	49·52	60·43
3. F. Y. M. (= 20 lbs. N)	881·64	—19·90	—4·26	47·64	58·14	3. F. Y. M. (= 20 lbs. N)	763·45	+23·05	+3·02	56·38	68·81
4. F. Y. M. (= 30 lbs. N)	950·00	+8·51	+0·90	49·98	60·99	4. F. Y. M. (= 30 lbs. N)	865·55	+40·05	+4·63	62·86	76·71
5. Rape-cake (= 20 lbs. N)	656·36	—5·08	—0·77	43·76	53·41	5. Rape-cake (= 20 lbs. N)	575·73	+8·67	+1·51	43·72	53·35
6. Amon. sulph (= 20 lbs. N)	470·45	—17·90	—3·80	43·13	52·64	6. Amon. sulph (= 20 lbs. N)	428·91	+2·01	+0·47	33·92	41·40
7. Pot. sulph	431·09	—22·99	—5·33	24·74	30·19	7. Pot. sulph	419·27	+2·31	+0·55	29·02	35·42
8. Superphos	925·00	—2·86	—0·31	47·01	57·37	8. Superphos	720·73	+21·30	+2·96	53·26	65·00
9. Pot. sulph + super	880·82	+3·22	+0·37	47·31	57·74	9. Pot. sulph + super	719·45	+6·85	+0·95	53·02	64·71
10. Amon. sulph + pot. sulph + superphos.	948·27	—1·10	—0·12	56·53	68·98	10. Amon. sulph + pot. sulph + superphos.	787·27	+38·40	+4·88	60·09	73·33
11. Legume crop	413·36	—2·46	—0·60	22·74	27·75	11. Legume crop	444·27	+3·95	+0·89	29·18	35·62
12. Green manure	622·18	—13·89	—2·23	27·90	34·04	12. Green manure	648·73	—5·66	—0·87	51·02	62·26
13. Deep-rooted pulse	473·91	—16·24	—3·43	24·68	30·12	13. Deep-rooted pulse	439·18	+7·19	+1·64	35·45	43·26
14. Deep + shallow root	355·18	+10·89	+3·07	15·24	18·60	14. Deep + shallow root	340·64	+20·57	+6·04	33·66	41·08
15. Green manure + pulse crop	683·18	—29·99	—4·39	52·55	64·13	15. Green manure + pulse crop.	687·55	+28·78	+4·19	57·27	69·89

Before we can attach any significance to these values of 'b', it is necessary to work out their standard errors and calculate the probability that they are really different from zero. If x and y are the two variates, b = the regression coefficient of y on x is given by

$$b = \frac{S\,(x-\bar{x})\,(y-\bar{y})}{S\,(x-\bar{x})^2} \qquad \text{. . . . . . (1)}$$

Fisher [1922] has shown that the distribution of

$$z = \frac{(b-\beta)\sqrt{S\,(x-\bar{x})^2}}{S\,\sqrt{(y'-y)^2}} \qquad \text{. . . . . . (2)}$$

(where β = population value of the regression coefficient and y' = estimated value of y) is given by the equation

$$df = \frac{1}{\sqrt{\pi}} \; \frac{\frac{n-3}{2}!}{\frac{n-4}{2}!} \; \frac{dz}{(1+z^2)^{\frac{n-1}{2}}} \qquad \text{. . . (3)}$$

so that, the distribution of z is the same as that of Fisher's t [1925]. Hence in order to test the significance of an observed b, all that we need calculate is the ratio z and find the probability P for $n'-2$ degrees of freedom where n' = number of observations.

The test can be conveniently carried out in practice with the help of the "Tables for Testing the Significance of Linear Regressions in the case of Time-Series and other Single-valued Samples" [Bose, 1934] published recently. The only thing necessary is to calculate $S\,(y-\bar{y})^2$ for the yields of each plot and divide this quantity by $S\,(x-\bar{x})^2 = 1/12\cdot n'(n'^2-1)$ where n' = number of years. The five per cent and one per cent values of 'b' corresponding to different values of $[S(y-\bar{y})^2]/[S(x-\bar{x})^2]$ are then obtained from the above-mentioned tables. Actual values are given in columns 5, 6, 11 and 12 of Tables I, II and III.

Discussion of results

It will be remembered that the fertilisers were applied direct to the maize crop while *arhar* and oats had only the residual effect of the manures. The deterioration in the yield of maize is therefore the more direct problem and will be considered first.

Deterioration of maize yield

The only treatment that prevented an appreciable deterioration in both blocks, A and B, is the third dose of farmyard manure containing thirty lbs. of nitrogen to the acre. The two smaller doses of ten lbs. and twenty lbs. nitrogen respectively showed practically identical deterioration of about eighteen lbs. per acre per year in block A and twenty-two lbs. per acre per year in block B. The untreated plots were not significantly worse so far as deterioration was concerned, although they gave significantly lower yields. The chemical fertilisers showed a falling off in yield between twenty lbs. and thirty lbs. per acre per year. It is worth noting that although the complete fertiliser combination (amon. sulph., pot. sulph. and superphos) gave the highest yield of maize, it also showed the greatest actual deterioration among the chemical fertilisers. With the legume and green-manure treatments, yields were poor, and the deterioration was also as high as from six to eight per cent.

Deterioration in arhar *and oats*

The yields of *arhar* and oats fluctuated considerably from year to year and it was not possible to graduate the trend by a straight line. On the available evidence it is not possible to say anything definite regarding deterioration of yields in *arhar* and oats under the given manurial treatments.

SUMMARY

(1) The yields of maize, *arhar* and oats in the Permanent Manurial and Rotation Experiments at Pusa from 1908-09 to 1929-30 have been analysed to find the amount of annual deterioration of each of these crops under different manurial treatments.

(2) A straight line has been fitted to the annual mean yields and the method of testing the significance of the slope has been discussed.

(3) It was observed that for maize, farmyard manure sufficient to contain thirty lbs. of nitrogen to the acre prevents deterioration of yield. Two smaller doses of farmyard manure containing ten lbs. and twenty lbs. of nitrogen per acre showed an average annual diminution of twenty lbs. per acre.

(4) Chemical fertilisers showed appreciable deterioration to the extent of twenty lbs. to thirty lbs. per acre.

(5) The complete fertiliser combination (amon. sulph. pot. sulph. and superphos enough to supply nitrogen, potash and phosphorus equal to the third dose of farmyard manure) exhibited the largest deterioration among the chemical fertilisers.

(6) Legume and green-manure treatments gave poor yields and considerably high annual diminutions.

(7) Oats and *arhar* received only the residual effect of the manure every year. From the observed yields, it is not possible to say anything definite regarding deterioration of their yields.

ACKNOWLEDGMENTS

We have great pleasure in acknowledging our indebtedness to Dr. W. McRae, late Director, Imperial Institute of Agricultural Research, Pusa, for his kind interest and suggestions during the present investigation. Our thanks are also due to Prof. P. C. Mahalanobis for his valuable advice and criticism in the preparation of this paper. To Mr. Anil Chandra Nag of the Statistical Laboratory, Calcutta, we are grateful for effective help during the reduction and analysis of data.

REFERENCES

Bose, S. S. (1934). *Sankhya* **1**, 277-288.

Bose, S. S. and Menon, T. V. G. (1937). *Ind. Jour. Agric. Sci.* **7**, 193-204.

Fisher, R. A. (1921). *Jour. Agric. Sci.* **11**, 107-135.

——— (1922). *Jour. Roy. Stat. Soc.* **85**, 597-612.

——— (1925). *Metron.* **5**, 90-104.

Royal Commission on Indian Agriculture (1928). Government of India, Delhi.

APPENDIX

On the method of testing the significance of an observed regression coefficient

Let us take the twenty-two yield figures of maize in the untreated plot of block A. The observed regression coefficient = — 22·28.

Sum of squares of yield $= S\,(y-y)^2 = 14{,}387{,}97$

Sum of squares of time (1 to 22) $= S(t-\bar{t})^2 = 1/12 \times 22 \times (22^2-1)$

$= 885 \cdot 5$

$$\therefore \frac{S_y{}^2}{S_x{}^2} = \frac{1438797}{885 \cdot 5} = 1624\,; \quad \frac{S_y}{S_x} \quad 40 \cdot 59$$

From the five per cent table of regression coefficient [Bose, 1934] we have for $n = 22$:—

$S_y{}^2/S_x{}^2$	S_y/S_x	b
0·15 0·20	0·3873 0·4472	0·1637 0·1890
1500 2000	38·73 44·72	16·37 18·90

The observed regression coefficient being greater than 18·90 (the 5 per cent value of b for $S_y{}^2/S_x{}^2 = 2000$) is significant on the five per cent level.

The five per cent point corresponding to $S_y{}^2/S_x{}^2 = 1624$ (or $S_y/S_x = 40 \cdot 59$) is easily obtained by simple interpolation.

ABSTRACT

Analysis of *Crotalaria juncea* with special reference to its use in green manuring and fibre production. B. N. SINGH and S. N. SINGH.
(*Jour. Amer. Soc. Agron.* **28**, *No.* 3, *March 1936*).

I. THE OUTLOOK AND THE PROCEDURE

Of all the green-manuring crops of India so far investigated the most important and the best suited to a wide range of both climatic as well as edaphic conditions is *Crotalaria juncea*—a fairly rapidly growing plant, with a relatively short life-cycle, capable of being raised without any special soil preparation. Its agricultural importance in improving the physical condition, water-holding capacity, and nutrient content of the soil on the one hand, and its industrial importance in yielding fibre on the other, have been detailed in the original paper. In spite of the high significance attached to this crop little is known about the nature and amount of material formed at successive stages of its growth, which constitutes the material for all subsequent changes after its incorporation into the soil.

This, in brief, is the outlook with which this series of investigations was instituted. The plant as a whole and its various parts were chemically analysed at successive intervals of a fortnight both for organic as well as inorganic constituents. On the basis of these observations the stage was located when the plant can yield the maximum amount of organic matter, contribute the most to the soil in terms of nitrogen and various fertilizing constituents, and provide the best quality of fibre.

II. SUMMARY OF RESULTS AND CONCLUSIONS

The entire life-cycle of the plant has been divided into four distinct physiological stages, *viz.*, (*i*) Juvenile (*ii*) Adolescent (*iii*) Partial senescent and (*iv*) Senescent. On the basis of these stages the results of investigation have been discussed.

Organic constituents.—The percentage of most of the organic constituents (Table 2A) runs parallel to the development of the plant during its early life-cycle (60-75 days)*. Some of the assimilate, *viz.*, reducing sugars and fats, behave otherwise, probably on account of the different physiological roles assigned to them. Reducing sugars decline throughout, while in fats this fall is interrupted by a rise near about the middle of the adolescent stage (45 days). Burying the crop at this stage or earlier than this means very little contribution to the soil and a failure of green-manuring practice.

---

*Age after germination.

On the other hand, total carbohydrates and sucrose which form a substantial fraction of the photosynthate, attain their maxima at the full adolescent stage (60 to 75 days) (Table 2A); while the celluloses and fats continue to rise till senescence. Although celluloses and fats form the major part of the organic matter, during senescent stage the general characters of the plant are very much changed, the stem becomes woody and hard and its decomposition would take a considerable time and irrigation, failing which incomplete decomposition may result in a severe attack by white ants.

Probably no single problem in agriculture transcends that of nitrogen. It is of outstanding importance because it is often present in insufficient amount or in unavailable form. The study of the nitrogen content of *Crotalaria* reveals that with the growth of the plant there is a gradual parallel increase in its percentage up to the later part of the adolescent stage (60 days) (Table 2A), a period when total carbohydrates also attain their maxima. The subsequent decline in its content after this stage, as well as its low value prior to this period, clearly indicates that ploughing under the crop before or after this critical stage would reduce the maximum manurial efficiency of the crop.

Inorganic constituents.—Like nitrogen, the ash percentage to dry matter increases gradually to reach the maximum value at its full adolescent stage (60 days) (Table 2A), after which it declines. On considering the individual elements certain fluctuations with regard to this stage are marked; but two very important inorganic plant components, *viz.*, calcium and potassium, like most of the organic constituents, show their highest accumulation when the initiation of the reproductive phase commences (60 to 75 days), and hence ploughing under the crop at this stage will return to the soil the maximum quantities of the inorganic nutrients so far drawn up from it.

On reviewing the data calculated per plant (Table 2B) and per acre for the entire plant (Table I) it is noticed that the maximum output of the majority of both organic and inorganic constituents is attained at the partial senescent stage (90 days). Ploughing under the mature woody plants at this stage will surely defeat the ultimate end in view, because only the quantity of the nutrients is not the true criterion of successful green-manuring. Moreover, the leaves and the roots which constitute no less an important fraction of the entire plant body contain the maximum quantity of most of these constituents only at the initiation of the reproductive phase (75 days). Whereas their percentage in dry matter in the entire plant and also plant parts is highest during the last fortnight of the adolescent stage (60 days) as summarized before. It is obvious that green-manuring, either with the entire plant or with tops and roots alone, at a stage coinciding with the initiation of the flowering primordia (75 days), should result in the maximum manurial value of this crop.

Manurial efficiency of plant parts.—The leaf appears to be the richest both in organic and inorganic constituents, while next in order are the root and stem. The utility of leaf and root for green-manuring was further tested by field experimentation. The beneficial effects of green-manuring with tops and roots is recognised in altering the colour and the texture of the soil and in the luxuriance in growth of the succeeding crop. The leaf containing simpler carbohydrates and proteins seems to be readily decomposed in the soil and contributes to the fertility of the land at an earlier date than the stem and root which contain the compounds of higher degree of complexities. The root stands midway between the two, both from the point of view of its rate of decomposition in the soil and also the percentage of the various individual fertilizing constituents contained therein. The greater manurial efficiency may be attributed to the leaf, and that the stem being less useful in this direction may well be utilized for other purposes. The root may be incorporated in the soil with the leaf to maintain the supply of the readily available nitrogenous compounds. Its slower rate of decomposition may also help the plant in overcoming the nitrogen deficiency, which may be felt at later stages of growth when the soil is relatively poor in nitrogen.

Fibre content.—The quantity of the fibre, as judged by the amount of celluloses in the dry material, increased continuously throughout the entire life-cycle of the plant (Table 2A & B). The stem contained the highest amount of the celluloses, the leaf the least. Field experiments revealed that the tonnage of the fibre was highest at the senescent stage (105 days), while the optimum results with regard to the qualities, *viz.*, length, tensile strength, and spinning value were obtained only at the adolescent stage (75 days).

On the basis of these observations it is concluded that the best period for green-manuring with *Crotalaria juncea* would be when the adolescent stage is about to finish and the reproductive phase commences. If both green-manuring and fibre production are the outlook the tops and roots could be ploughed in for green-manuring and stems used for fibre production when the plant is in its full adolescent stage (75 days).

TABLE I

Fertilizing constituents in lbs. per acre to be added to the soil by incorporating the plant into the soil at successive stages of its life-cycle

Age in days	Samples	Total roughage	Reducing sugars	Fats	Total carbohydrates	Sucrose	Celluloses	Nitrogen	Ash	Calcium	Potassium	Phosphorus	Magnesium	Sulphur
	Juvenile (young) stage													
1	Tops and roots entire	7·9 11·5	0·60 0·86	1·08 1·32	1·60 2·10	0·26 0·33	0·36 0·99	0·54 0·72	0·62 0·79	0·16 0·19	0·05 0·06	0·03 0·03	0·03 0·04	0·12 0·19
15	Tops and roots entire	20·2 28·8	1·47 2·01	2·67 3·10	5·44 6·63	0·92 1·15	2·11 3·16	1·58 1·94	1·98 2·27	0·46 0·56	0·28 0·29	0·11 0·12	0·08 0·09	0·62 1·02
30	Tops and roots entire	248·1 380·5	18·00 25·20	30·30 35·10	69·90 89·50	12·04 17·20	13·30 50·90	22·40 27·60	32·70 37·80	9·30 10·70	3·97 4·31	1·44 1·65	0·86 1·23	10·39 11·25
	Adolescent stage													
45	Tops and roots entire	828·6 1165·3	51·8 64·1	46·1 106·0	227·1 338·1	47·8 62·4	59·7 191·1	81·2 105·2	108·5 122·2	42·6 43·8	22·80 23·29	2·79 4·05	3·39 4·21	28·11 37·55
60	Tops and roots entire	3451·6 6850·8	175·7 267·9	467·4 676·3	1204·5 2064·2	226·3 480·6	282·2 1376·9	396·2 683·8	601·3 962·5	219·2 332·9	131·30 202·52	13·33 21·32	20·33 21·25	137·54 168·11
75	Tops and roots entire	5362·9 9933·5	216·3 313·5	745·1 1055·2	1687·1 2837·1	417·7 720·8	489·2 2153·1	473·9 781·1	840·6 1204·9	276·1 431·4	188·30 309·94	15·71 28·51	35·88 51·95	178·04 190·71
	Partial senescent stage													
90	Tops and roots entire	5782·1 12841·3	231·6 369·3	954·2 1607·8	1649·6 3342·9	362·3 739·9	433·3 2863·3	447·3 783·9	573·8 1351·1	232·7 502·1	197·67 424·85	15·94 34·06	40·46 71·41	130·00 337·60
	Senescent stage													
105	Tops and roots entire	3846·4 15180·3	41·4 151·9	629·7 2220·9	906·9 3055·8	145·9 509·5	300·9 3350·5	158·4 473·8	261·5 1264·2	31·3 170·6	105·86 455·43	8·48 31·91	10·46 43·59	46·68 169·52

TABLE II

Age in days	Reducing sugars	Fats	Total carbohydrates	Sucrose	Celluloses	Nitrogen	Ash	Calcium	Potash	Phosphorus	Magnesium	Sulphur
A. The percentage of the various fertilizing constituents in the entire plant of *Crotalaria juncea* at successive stages of its life-cycle												
1 . . .	7·53	11·53	18·51	3·12	8·61	6·32	6·87	1·73	0·52	0·30	0·35	1·95
15 . . .	7·01	10·85	23·12	4·02	11·05	7·05	8·01	2·04	1·00	0·39	0·30	2·42
30 . . .	6·63	9·50	23·53	4·53	13·46	8·12	9·95	2·83	1·13	0·43	0·29	2·96
45 . . .	5·50	9·01	29·01	5·35	16·39	9·03	10·56	3·76	2·00	0·35	0·36	3·22
60 . . .	3·91	9·87	30·13	7·01	20·09	9·98	14·05	4·86	2·95	0·31	0·41	2·45
75 . . .	3·15	10·62	28·56	7·25	21·67	7·86	12.13	4·34	4·12	0·28	0·52	1·92
90 . . .	2·87	12·52	26·03	5·76	22·29	6·31	10·52	3·91	3·54	0·26	0·55	1·85
105 . . .	1·00	14·63	20·13	3·35	24·17	3·12	8·32	1·12	3·00	0·21	0·28	0·72
B. Amount per plant in grams of the various fertilizing constituents in the entire plant												
1 . . .	0·0026	0·0040	0·0064	0·0010	0·0030	0·0022	0·0024	0·0006	0·0001	0·0001	0·0001	0·0006
15 . . .	0·0061	0·0094	0·0201	0·0035	0·0096	0·0062	0·0069	0·0017	0·0008	0·0003	0·0002	0·0031
30 . . .	0·0765	0·1095	0·2713	0·0522	0·1551	0·0837	0·1147	0·0326	0·0130	0·0050	0·0034	0·0341
45 . .	0·1944	0·3182	1·0244	0·1891	0·5789	0·3189	0·3720	0·1328	0·0706	0·0123	0·0127	0·1138
60 . . .	0·8121	2·0496	6·2551	1·4563	4·1726	2·0721	2·9168	1·0089	0·6137	0·0646	0·0856	0·5094
75 . . .	0·9500	3·1976	8·5973	2·1842	6·5245	2·3670	3·6514	1·3067	1·2402	0·0865	0·1574	0·5779
90 . . .	1·1180	4·8722	10·1300	2·2422	8·6768	2·3756	4·0942	1·5215	1·2875	0·1032	0·2164	0·7200
105 . . .	0·4605	6·7300	9·2600	1·5439	10·1532	1·4356	3·8307	0·5168	1·3801	0·0967	0·1321	0·5137

RECENT PUBLICATIONS OF THE IMPERIAL AGRICULTURAL BUREAUX

I. OBTAINABLE FROM THE IMPERIAL BUREAU OF SOIL SCIENCE, ROTHAMSTED EXPERIMENTAL STATION, HARPENDEN, HERTS

Periodical Abstracts

Lists of publications and papers on Soil Science published in the Empire Overseas in :—

	Price.	
	s.	*d.*
1933	1	0
1934	1	0
Soil Research in the British Empire published during 1935	1	0
Lists of Publications relating to Soils and Fertilisers—		
Published monthly, per annum, post free	10	0
Monthly Letters—		
Free to recipients, within the British Empire, of "Publications relating to Soils and Fertilisers." Subscription, outside the Empire, per annum.	4	0
Recent Developments in Soil Analysis—		
Quarterly Supplement to the above publications. Separate copies each	0	6

Occasional Papers

Technical Communications—		
32. Tea Soils (by H. H. Mann)	2	0
33. Organic Manures (by S. H. Jenkins)	2	0
34. Tropical Soils in relation to Tropical Crops	2	6
Annual Report : For the year 1933-34	0	6
Annual Report : For the year 1934-35	0	6
Bibliographies—		
Bibliography on Coffee	2	0
Catalogue of Journals and Periodicals in the Library of Rothamsted Experimental Station	2	
Special Publication—		
The Katamorphism of Igneous Rocks under Humid Tropical Conditions (by the late Sir J. B. Harrison)	5	0
Bibliography of Soil Science, Fertilizers and General Agronomy, 1931-34	25	0

II. OBTAINABLE FROM THE IMPERIAL BUREAU OF ANIMAL HEALTH, VETERINARY RESEARCH LABORATORY, NEW HAW, WEYBRIDGE, SURREY

Abstracting Journal

The Veterinary Bulletin—		
1931. Vol. 1. Quarterly (1st Number, April)	7	6
Annual Subscription	20	0
Subsequent Volumes. Monthly (1st Number, January)	5	0
Annual Subscription (postage paid)	40	0

Indexing Publication

	Price *s.*	 *d.*
Index Veterinarius—Four issues a year. First issue April 1933— Annual Subscription (postage paid). Volumes I to III mimeographed, Volume IV onwards printed	100	0

III. OBTAINABLE FROM THE IMPERIAL BUREAU OF ANIMAL NUTRITION, ROWETT RESEARCH INSTITUTE, BUCKSBURN, ABERDEEN

Journal

Nutrition Abstracts and Reviews. (Issued under the direction of the Imperial Agricultural Bureaux Council, the Medical Research Council and the Reid Library.)—		
Subscription per Volume of 4 numbers	42	0
Per single number	13	0

Occasional Papers

Technical Communications—		
6. The Composition of Certain African Foods and Feeding Stuffs	1	0
7. Wheat : Pre-eminence as a Cereal Food, Nutritive Value ; Relation to Health and Disease	1	0
Occasional Communications—		
1. The Effect of Climate on the Composition of Pasture Plants	..	

IV. OBTAINABLE FROM THE IMPERIAL BUREAU OF PLANT GENETICS (FOR CROPS OTHER THAN HERBAGE), PLANT BREEDING INSTITUTE, SCHOOL OF AGRICULTURE, CAMBRIDGE

Journal

Plant Breeding Abstracts—		
Quarterly. Annual Subscription	15	0
Single copies	5	0

Occasional Papers

Indexes to Plant Breeding Abstracts—		
Subject Index to Vols. I to V of Plant Breeding Abstracts	5	0
Supplements to Plant Breeding Abstracts—		
Summary of Reports received from Countries exclusive of the British Empire, 1928-31. Supplement I	2	6
Summary of Reports received from Stations in the British Empire, 1932-1935. Supplement II	5	0
Technical Communications—		
Vernalization and Phasic Development of Plants (Joint Publication of the Imperial Bureaux of Plant Genetics)	10	0
Bibliographical Monographs—		
The Experimental Production of Haploids and Polyploids	5	0
Breeding Resistant Varieties, 1930-33 (Supplement)	2	0

V. OBTAINABLE FROM THE IMPERIAL BUREAU OF PLANT GENETICS (HERBAGE PLANTS), WELSH PLANT BREEDING STATION, AGRICULTURAL BUILDINGS, ALEXANDRA ROAD, ABERYSTWYTH, WALES

Journals

	Price s.	d.
Herbage Abstracts—		
Quarterly. Annual Subscription	15	0
Single numbers	5	0
Herbage Reviews—		
Subscription is at present—Vol. 1 (1933), Vol. 2 (1934), Vol. 3 (1935), Vol. 4 (1936)—included in that to Herbage Abstracts		

Occasional Papers

	s.	d.
Bulletins—		
15. Grassland and Forage Crops in Thuringia, Czechoslovakia and Hungary, August, 1934	3	6
17. Vernalization and Phasic Development of Plants. December, 1935. (Joint publication of the Imperial Bureaux of Plant Genetics)	10	0

VI. OBTAINABLE FROM THE IMPERIAL BUREAU OF FRUIT PRODUCTION, EAST MALLING RESEARCH STATION, EAST MALLING, KENT

Journal

	s.	d.
Horticultural Abstracts—		
A quarterly abstract publication of current horticultural literature—		
Annual Subscription	15	0
Single copy	4	0

Technical Communications

	s.	d.
6. The Nutrition and Manuring of Soft Fruits. 1936. T. Wallace	2	0
7. Vegetative Propagation of Tropical and Sub-tropical Fruits. 1936. J. St. Clair Feilden and R. J. Garner	2	0

Occasional Papers

	s.	d.
3. Annotated Bibliography on Bitter-Pit. 1934	1	6
Other Publications—		
Index to Volumes I—X of the *Journal of Pomology and Horticultural Science*, 1933. Compiled by Bureau, published by the Editors of the *Journal of Pomology and Horticultural Science*. Available from the Bureau	5	0
Old and New standpoints on senile degeneration, 1931. By A. P. C. Bijhouwer	0	6

VII. OBTAINABLE FROM THE IMPERIAL BUREAU OF ANIMAL GENETICS, INSTITUTE OF ANIMAL GENETICS, UNIVERSITY OF EDINBURGH, KING'S BUILDINGS, WEST MAINS ROAD, EDINBURGH

Journal

	s.	d.
Animal Breeding Abstracts (Quarterly) Commencing April, 1933. Annual Subscription	15	0

Oasccional Papers

	Price s.	d.
The Technique of Artificial Insemination, 1933	2	6
Bibliography of the works of J. C. Ewart (free to subscribers of Animal Breeding Abstracts, Vol. 1), 1934	0	6
Animal Breeding in the British Empire. A Survey of Research and Experiment, 1934	2	0

VIII. OBTAINABLE FROM THE IMPERIAL BUREAU OF AGRICULTURAL PARASITOLOGY, INSTITUTE OF AGRICULTURAL PARASITOLOGY, WINCHES FARM DRIVE, HATFIELD ROAD, ST. ALBANS, HERTS

Journals

	s.	d.
Bibliography of Helminthology. For the year 1933—		
Library Edition, bound in cloth	10	6
Stiff paper cover only	8	0
Helminthological Abstracts. (Issued in five or six parts per volume, each volume, covering the literature of a single year.) Subscription per volume, payable in advance	30	0
Completed volumes bound in cloth	32	0

Occasional Papers

	s.	d.
(*g*) The Pathology and Aetiology of Plant Lesions caused by Parasitic Nematodes. By. T. Goodey, 34 pp.	5	0
(*h*) The Bearing of the Physiology of Parasitic Nematodes on their Treatment and Control. By A. Lapage. 21 pp.	3	0
Notes and Memoranda—		
11. Recent Developments in the Control of *Heterodera marioni*, 6 pp.	1	6

PUBLICATIONS ISSUED BY THE IMPERIAL INSTITUTE OF ENTOMOLOGY, 41, QUEEN'S GATE, LONDON, S. W. 7

Bulletin of Entomological Research.—Published quarterly and containing original articles on Economic Entomology. Issued post-free.

	s.	d.
Annual subscription (payable in advance)	30	0

Review of Applied Entomology.—Consisting of abstracts or reviews of all current literature on Economic Entomology throughout the world. Published monthly in two series. Series 'A' dealing with Insect and other Arthropod pests of cultivated plants, forest trees and stored products of animal and vegetable origin, Series 'B' dealing with insects, ticks, etc., conveying disease or otherwise injurious to man and animals. Issued post-free.

	Series 'A' s.	Series 'B' s.
Annual subscription (payable in advance)	30	15
Vol, XXIII (1935)	42	21

	Price	
	s.	*d.*
Zoological Record—Part Insecta.—Published annually about July in each year and containing as complete a record as possible of the literature of the previous year, chiefly from the systematic standpoint.		
Annual subscription (including postage)	15	6
Report of the Fourth Imperial Entomological Conference, 19-27th September 1935	4	0

PUBLICATIONS OBTAINABLE FROM THE IMPERIAL MYCOLOGICAL INSTITUTE, KEW, SURREY

Journal

	s.	*d.*
Review of Applied Mycology.—Annual subscription, 12 monthly parts, with title page and index (post-free)	21	0
Single part	2	0
Title page and index	3	0
Report on the Third Imperial Mycological Conference, 1934 . . .	2	0

APPENDIX

INSTRUCTIONS TO AUTHORS OF PUBLICATIONS OF THE IMPERIAL COUNCIL OF AGRICULTURAL RESEARCH*

1. All manuscripts should be clean, clear and carefully revised. Only one side of the paper should be used, and as far as practicable the original type-written copy and not a carbon copy should be sent. Capitals should be sparingly used, and all the necessary punctuation should be done in the MS. and not left for introduction in proofs.

2. The title of a paper should not be lengthy.

3. It is desirable that the MS. should have suitable heads and sub-heads. In numbering the principal divisions of a paper roman numerals should be used. The use of arabic figures and (*a*), (*b*), (*c*), etc., is generally reserved for numbering the sub-divisions coming under each head.

4. Articles submitted for publication either in the " Indian Journal of Agricultural Science " or in the " Indian Journal of Veterinary Science and Animal Husbandry " should be accompanied by abstracts for publication in " Agriculture and Live-stock in India ". Abstracts should be concise, but should be long enough to explain the matter dealt with ; ordinarily no abstract should exceed 200 words.

5. When a word or line is intended to be printed in *italics* it should be underlined with a single line, in SM. CAP. with two lines, in CAPITALS with three lines, and when in **antique** (heavy type) with a wavy line (〰〰〰).

6. In descriptive matter, numbers under 100 and all numbers occurring at the beginning of a sentence should be in words.

7. Local names for crops, technical operations, etc., should be defined where they first occur in the text, *e.g.*, *rabi* (spring crop). The use of local weights and measures should be avoided as far as possible. Vernacular names, such as *jowar*, *bajri*, should be in italics without a capital letter, and each such name where it first occurs should be followed by its scientific equivalent in brackets, *e.g.*, *jowar* (*Andropogon Sorghum*). It is usual to write the initial letters of varietal names in capitals, *e.g.*, Striped Mauritius, Dharwar-American cotton and Broach cotton.

8. Botanical and zoological names are printed in italics and should be underlined in the MS., *e.g.*, *Triticum vulgare* L. ; *Diplodia Corchori* Syd. ; *Pyrilla aberrans* Kirby. The International Rules of Botanical Nomenclature and the International Rules of Zoological Nomenclature should be followed. The names of chemical substances should not be written with a capital letter ; they are printed in roman types (*e.g.*, calcium carbonate, prussic acid).

---

* Spare copies of these Instructions can be had on application to the Secretary, Imperial Council of Agricultural Research (Publication Section), New Delhi.

9. The following and similar abbreviations may be used freely :—*viz., e.g., i.e.,* mm. (millimetre), cm. (centimetre), grm. (gramme), mg. (milligramme), c. c. (cubic centimetre), sp. gr. (specific gravity), lb. (pound), cwt. (hundredweight), in. (inch), ft. (foot), oz. (ounce), md. (maund), sr. (seer), ch. (chattak). Other abbreviations should be used sparingly, if at all.

10. References to plates should be given within brackets, without prefixing the word " see " or " cf." in the MS. itself, and should not be left over for introduction in proofs. For example. " The parasite (Pl. X, fig. 4) was present late in 1906 ".

11. The word " Table " is preferable to " Statement ", and tables should be numbered consecutively in roman figures. Each table should have an explanation as a sub-head. It is more convenient for reference if tables can be printed horizontally ; for this purpose they should not exceed in width the printing measure of the page (5 in.). *Example—*

TABLE IV

Results of water-saving experiments on wheat (Pusa 12) at Gungapur, Haripur and Sargodha, 1916 17

Station	No. of irrigations including the preliminary watering	YIELD PER ACRE IN MAUNDS AND SEERS				AVERAGE YIELD PER ACRE			
		Grain		Straw		Grain		Straw	
		Mds.	Srs.	Mds.	Srs.	Mds.	Srs.	Mds.	Srs.
Gungapur . . .	One	12	19½	20	10	9	34	21	17
Haripur . . .	,,	8	31	19	14				
Sargodha . . .	,,	8	12½	25	27½				

12. References to literature, arranged alphabetically according to authors' names, should be placed at the end of the article, the various references to each author being arranged chronologically. Each reference should contain the name of the author (with initials), the year of publication, the abbreviated title of the publication, volume and page. In the text the reference should be indicated by the author's name followed by the year of publication enclosed in brackets ; when the author's name occurs in the text, the year of publication only need be given in brackets. If reference is made to several articles published by one author in a single year, these should be numbered in sequence and the number quoted after the

year both in the text and in the collected references. This system of referencing is the same as is used in the "Biochemical Journal" with slight modification and will be clear from the following illustration :—

The work of Osborne and Mendel [1919, 1, 2] and Steenbock and Boutwell [1919] had indicated an association of the fat-soluble vitamin with the green parts of plants. This view was examined by Coward and Drummond [1921], who reported that vitamin A was not synthesised by etiolated shoots but that green leaves were active in its formation. Another worker [Wilson, 1922], on the other hand, found that etiolated shoots if given in sufficient quantity could supply the fat-soluble vitamin and that this factor was therefore formed in the absence of light.

REFERENCES

Coward, K. H. and Drummond, J. C. (1921). *Biochem. J.* **15**, 530.
Osborne, T. B. and Mendel, L. B. (1919, 1). *J. Biol. Chem.* **37**, 187.
———————— (1919, 2). *J. Biol. Chem.* **41**, 549.
Steenbock, H. and Boutwell, R. (1919). *J. Biol. Chem.* **41**, 149.
Wilson, J. (1922). *J. Biol. Chem.* **51**, 455.

Abbreviations, as far as possible, should follow the system adopted in "A World List of Scientific Periodicals" published by the Oxford University Press.

13. Papers should be complete when submitted for publication. As alterations and additions at the proof stage cause both additional expense and delay, they should be resorted to as little as possible. In making corrections in proofs the recognized symbols which will be found in the "Standard Dictionary" should be used. Second (page) proofs will be submitted to authors who should return them promptly.

Illustrations

14. As the *format* of the journals has been standardized, the size adopted being crown quarto (about 7½ in. × 9⅝ in. cut), no text-figure, when printed, should exceed 4½ × 5 inches. Figures for plates should be so planned as to fill a crown quarto plate—the maximum space available for figures being 5¾ × 8 inches exclusive of that for letterpress printing.

15. Photos or drawings for illustration should accompany the manuscript and each should bear on the reverse side the name of the paper to which it relates together with the title or legend, figure or plate number, and the size to be reproduced. When giving instructions for reduction linear measurements are understood ; thus, "half-size" means reduce to half the length and breadth, not half the area. A photograph should not be rolled up, nor pinned, and should always be packed flat. A complete list of plates and figures should always accompany the paper.

16. Line drawings should be made with clear black lines on smooth white paper, preferably Bristol board. Rough paper should be avoided. Care should be taken that all the lines are drawn firmly ; scratchy or grey lines, produced by the ink

being thinned down, are not permissible. Drawings should be larger than the required size. All lettering should be neatly and clearly put in, care being taken to make all lettering sufficiently large to stand reduction.

17. For half-tone work, copy should be made on glossy silver paper and of the same size or larger than the size required.

18. For three-colour work, copy may be oil painting, water-colour, coloured photograph or coloured transparency, and larger than the size required. In preparing copy, one should use only the primary colours, in any combination, as only inks of primary colours are used in printing. Originals can be enlarged, if necessary, but this should be avoided if possible.

19. For detailed instructions regarding preparation of illustrations, it would be of advantage to refer to Mr. C. M. Hutchinson's article on " Photographic Illustrations " in the *Agricultural Journal of India*, Vol. XI, Pt. 3, July 1916, and Mr. A. W. Slater's paper on " The Preparation and Reproduction of Scientific Illustrations " in the *Proceedings of the Third Entomological Meeting*, 1919, which has been reprinted as *Bulletin No. 114 of the Agricultural Research Institute, Pusa.*

PUBLICATIONS OF THE IMPERIAL COUNCIL OF AGRICULTURAL RESEARCH, INDIA

1. Agriculture and Live-stock in India

A bi-monthly Journal of Agriculture and Animal Husbandry for the general reader interested in Agriculture or Live-stock in India or the Tropics. (Established 1931. Published in January, March, May, July, September and November. Prepayable subscription Rs. 6 or 9*s.* 9*d.* per annum inclusive of Indian postage. Price per part Rs. 2 or 3*s.* 6*d.* inclusive of Indian postage). Volumes I to VI complete are available.

2. The Indian Journal of Agricultural Science

A bi-monthly Scientific Journal of Agriculture and the allied sciences, mainly devoted to the publication of the results of original research and field experiments. (Established 1931. Published in February, April, June, August, October and December. Prepayable subscription Rs. 15 or 24*s.* per annum inclusive of Indian postage. Price per part Rs. 3 or 5*s.* 3*d.* inclusive of Indian postage.) Volumes I to VI complete are available.

3. The Indian Journal of Veterinary Science and Animal Husbandry

A quarterly Journal for the publication of scientific matter relating to the health, nutrition and breeding of live-stock. (Established 1931. Published in March, June, September and December. Prepayable subscription Rs. 6 or 9*s.* 9*d.* inclusive of Indian postage. Price per part Rs. 2 or 3*s.* 6*d.* inclusive of Indian postage.) Volumes I to VI complete are available.

4. Scientific Monographs of the Imperial Council of Agricultural Research

NCAG—SM/1	No. 1. The Fungi of India, By E. J. Butler, C.I.E., D.Sc., M.B., F.R.S. and G. R. Bisby, Ph.D. (1931). Price Rs. 6-12-0 or 11*s.* (As. 8.)
NCAG—SM/2	No. 2. Life-histories of Indian Microlepidoptera, Second Series: Alucitidae (Pterophoridae, Tortricina and Gelechiadae), By T. Bainbrigge Fletcher, R.N., F.L.S., F.E.S., F.Z.S. (1932). Price Rs. 3-4-0 or 5*s.* 6*d.* (As. 6.)
NCAG—SM/3	No. 3. The Open Pan System of White-Sugar Manufacture, By R. C. Srivastava, B.Sc. (1935, 2nd edition). Price Rs. 3-2-0 or 5*s.* 6*d.* (As. 9.)
NCAG—SM/4	No. 4. Life-histories of Indian Microlepidoptera: Cosmopterygidae to Neopseustidae, By T. Bainbrigge Fletcher, R.N., F.L.S., F.E.S., F.Z.S. (1933). Price Rs. 4-8-0 or 7*s.* 6*d.* (As. 8.)
NCAG—SM/5	No. 5. The Bombay Grasses, By E. J. Blatter, S.J., Ph.D., F.L.S. and C. McCann, F.L.S. Illustrated by R. K. Bhide. Price Rs. 20-12-0 or 32*s.* 6*d.*
NCAG—SM/6	No. 6. Helminth Parasites of the Domesticated Animals in India, By G. D. Bhalerao, M.Sc. Price Rs. 7-12-0 or 13*s.* 3*d.*
NCAG—SM/7	No. 7. Influence of Manures on the Wilt Disease of *Cajanus indicus* Spreng. and the Isolation of Types Resistant to the Disease, By W. McRae, M.A., D.Sc. (Edin.), F.L.S. and F. J. F. Shaw, D.Sc. (Lond.), A.R.C.S., F.L.S. (1933). Price Rs. 2-4-0 or 4*s.* 3*d.* (As. 5.)

NCAG—SM/8	No. 8. The Silk Industry of Japan with Notes on Observations in the United States of America, England, France and Italy, By C. C. Ghosh, B.A., F.E.S. (1933). Price Rs. 4 or 6*s*. 9*d*. (As. 7).
NCAG—SM/9	No. 9. Mechanical Cultivation in India. A History of the Large Scale Experiments carried out by Burmah-Shell Oil Storage and Distributing Company of India, Ltd., By C. P. G. Wade. Price Rs. 3-14-0 or 6*s*. 6*d*.
NCAG—SM/10	No. 10. Spotted Boll-worms of Cotton (*Earias fabia* Stoll and *Earias insulana* Boid.) in South Gujarat, (Final report on investigations financed by the Indian Central Cotton Committee, 1923-1931), By B. P. Deshpande, M.Ag. and N. T. Nandkarny. Price Rs. 5-14-0 or 9*s*. 6*d*.
NCAG—SM/11	No. 11. Investigations on the Course and Distribution of the Nerves supplying Levator anguli scapuli and Rhomboideus Muscles and the Formation of the Phrenic Nerve in the Ox, with Observations on certain Anatomical Deviations, By H. N. Chelva Ayyangar, G.M.V.C. (*In the Press*).

5. Miscellaneous Bulletins of the Imperial Council of Agricultural Research

NCAG—MB/1	No. 1. List of Publications on Indian Entomology (1930), Compiled by the Imperial Entomologist, Pusa. Price As. 14 or 1*s*. 6*d*.
NCAG—MB/2	No. 2. List of Publications on Indian Entomology (1931), Compiled by the Imperial Entomologist, Pusa. Price As. 8 or 10*d*.
NCAG—MB/3	No. 3. List of Publications on Indian Entomology (1932), Compiled by the Imperial Entomologist, Pusa. Price As. 12 or 1*s*. 3*d*.
NCAG—MB/4	No. 4. Host Plant Index of Indo-Ceylonese Coccidae, By S. Ramachandran, L.Ag. and T. V. Ramakrishna Ayyar, B.A., Ph.D., F.Z.S. Price Rs. 1-10 or 2*s*. 9*d*.
NCAG—MB/5	No. 5. List of Publications on Indian Entomology (1933), Compiled by the Officiating Imperial Entomologist, Pusa. Price As. 9 or 1*s*.
NCAG—MB/6	No. 6. Bee-keeping, By C. C. Ghosh, B.A., F.E.S. (3rd Edition.) Price Rs. 1-14 or 3*s*. 3*d*.
NCAG—MB/7	No. 7. List of Publications on Indian Entomology (1934), Compiled by the Officiating Imperial Entomologist and the Imperial Entomologist, Pusa. Price Rs. 1-2-0 or 2*s*.
NCAR—MB/8	No. 8. Selected Clinical Articles, By M. G. K. Sharma, G.P.V.C. and R. L. Kaura, B.V.Sc., M.R.C.V.S. Price As. 8 or 10*d*.
NCAR—MB/9	No. 9. Statistical Methods and their Application to Agronomy: A Bibliography, By K. K. Guha Roy, B.A. Price Rs. 2-2 or 4*s*.
NCAR—MB/10	No. 10. Diseases of Sugarcane and Methods for their Control, By L. S. Subramaniam. Price Rs. 1-14-0 or 3*s*. 3*d*.
NCAR—MB/11	No. 11. Tables of Standard Errors of Mendelian Ratios, By Swarn Singh Purewal, and P. Krishna Rao. Price As. 12 or 1*s*. 3*d*.
NCAR—MB/12	No. 12. List of Publications on the Botany of Indian Crops, Part II, for the period 1928-32, By R. D. Bose. Price Rs. 3-6-0 or 5*s*. 9*d*.
NCAR—MB/13	No. 13. Two New Statistical Tables based upon Fisher's '*t*', By M. Vaidyanathan. Price As. 6 or 8*d*.
NCAR—MB/14	No. 14. List of Publications on Indian Entomology, Compiled by the Imperial Entomologist. (*In the Press*).

6. Annual Report of the Imperial Council of Agricultural Research

NCAG—AR/31	Annual Report of the Imperial Council of Agricultural Research for the years 1929-30 and 1930-31. Price As. 12 or 1*s*. 3*d*. (Anna 1.)
NCAG—AR/32	Annual Report of the Imperial Council of Agricultural Research for the year 1931-32. Price As. 6 or 8*d*. (As. 2.)
NCAG—AR/33	Annual Report of the Imperial Council of Agricultural Research for the year 1932-33. Price As. 6 or 8*d*.
NCAG—AR/34	Annual Report of the Imperial Council of Agricultural Research for the year 1933-34.
NCAG—AR/35	Annual Report of the Imperial Council of Agricultural Research for the year 1934-35.
NCAR—AR/36	Annual Report of the Imperial Council of Agricultural Research for the year 1935-36.

7. Agriculture and Animal Husbandry in India (Formerly called Review of Agricultural Operations in India)

NCAG—R.1/29	Review of Agricultural Operations in India, 1928-29. Price Rs. 3-2-0 or 5*s*. 6*d*. (As. 8.)
NCAG—R.1/31	Review of Agricultural Operations in India, 1929-31. Price Rs. 5 or 3*d*. (As. 10.)
NCAG—R.1/33	Review of Agricultural Operations in India, 1931-33. Price Rs. 5-12-0 or 9*s*. 6*d*.
NCAG—R.1/35	Agriculture and Animal Husbandry in India : Part I—Crop Production. Price Rs. 4-14-0 or 8*s*. Part II—Animal Husbandry. Price Re. 1 or 1*s*. 9*d*.

8. Proceedings of the Board of Agriculture and Animal Husbandry

NCAG—P. 1	Proceedings of the First Meeting of the Animal Husbandry Wing of the Board of Agriculture and Animal Husbandry held at New Delhi from the 20th to 23rd February 1933, with Appendices. Price Rs. 5-14-0 or 9*s*. 6*d*.
NCAR—P. 2	Proceedings of the First Meeting of the Crops and Soils Wing of the Board of Agriculture and Animal Husbandry in India held at Delhi from the 25th February to the 2nd March 1935, with Appendices. Price Rs. 6 or 9*s*. 9*d*.

A description of the Imperial Institute of Veterinary Research, Muktesar, and its Sub-station, the Imperial Veterinary Serum Institute, Izatnagar, By F. Ware, F.R.C.V.S. Price Rs. 1-4-0 or 2*s*.

The Production of Cigarette Tobacco by Flue-curing, By F. J. F. Shaw, C.I.E., D.Sc., A.R.C.S., F.L.S. and Kashi Ram. *Imp. Inst. Agri. Res. Pusa Bull.* No. 187. Reprinted (1935). Price Re. 1 or 1*s*. 9*d*.

A Handbook of Statistics for use in Plant Breeding and Agricultural Problems, By F. J. F. Shaw, C.I.E., D.Sc., A.R.C.S., F.L.S. Price Rs. 4-6-0 or 7*s*. 3*d*.

[Copies of the above publications can be had from :—

The Manager of Publications, Civil Lines, Delhi, India.

In Europe and America.—From Public Department (Publications Branch), Office of the High Commissioner for India, India House, Aldwych, London, W.C. 2.]

CORRIGENDUM

Indian Journal of Agricultural Science, Vol. VI, Part III (June 1936)

Pages 726-733 : Figures 1-8 refer respectively to control, $CoCl_2$, ICl, $ZnCl_2$, $AlCl_3$, $MnCl_2$, $LiCl$, and $NaCl$.

GIPD--524 ICofAR—S3—3-3-37—800.

ORIGINAL ARTICLES

THE CLASSIFICATION OF THE COTTONS OF ASIA AND AFRICA

BY

J. B. HUTCHINSON

AND

R. L. M. GHOSE

Institute of Plant Industry, Indore, Central India

(Received for publication on 24th August 1936)

(With Plates XII—XIV and twenty-eight text-figures)

CONTENTS

PAGE
I. INTRODUCTION . . . 233
II. REVIEW OF LITERATURE . . . 234
III. CLASSIFICATION: . . . 236
    Evaluation of characters . . . 236
    Key to species . . . 242
    Description of species . . . 245
IV. DISCUSSION . . . 251
V. SUMMARY . . . 255
VI. ACKNOWLEDGMENTS . . . 256
REFERENCES . . . 257

I. Introduction

THE need for a satisfactory classification of Asiatic cottons has long been felt in India, and a considerable body of literature has been published on the subject. All workers from the time of Gammie and Watt have been largely influenced by the demand of agricultural botanists for a satisfactory agricultural classification. It is the opinion of the present authors that the resulting combination of botanical principles and agricultural convenience is responsible for a great deal of confusion.

The present paper is written in the belief that sufficient information is now available from studies of genetics and plant geography to enable a satisfactory classification to be devised on botanical principles only.

The paper is primarily intended for Indian workers, but the facts that the indigenous cottons of Africa are closely related to those of India, and that the exotic types acclimatised there are of the same species as those acclimatised in India, have made it convenient to deal with the two continents together. Only indigenous types are dealt with in detail. Descriptions of exotic types follow Harland [1932] closely, and are included for the convenience of workers who have to deal with acclimatised cottons alongside indigenous types.

II. Review of literature

The more important of the older contributions to the classification of the Asiatic section of the genus *Gossypium* are those of Gammie [1907], Watt [1907], and Leake and Ram Prasad [1914]. Watt supplemented his earlier work by two short papers [Watt, 1926 ; 1927]. More recently Zaitzev [1928] has surveyed the ecology, geographical distribution and classification of the genus, and Harland [1928 ; 1932 ; 1936] has discussed its classification and the state of our knowledge of its genetics. The present work is based on the senior author's classification of the Asiatic cottons included in Harland's [1932] monograph, and the descriptions of exotic (American) cottons follow Harland closely.

Gammie [1907] was of the opinion that the cultivated Asiatic cottons all belong to a single true species, *G. obtusifolium*, with two main sub-species, *G. arboreum* and *G. herbaceum*. He described the types of Indian cotton in great detail in the hope of meeting the need of economic botanists for an agricultural classification. He recognized the following characters as important : habit of plant and arrangement of branches, shape and hairiness of leaves and bracteoles, colour of flowers, boll size and lint characters. Many of his sub-divisions appear to be based on agricultural rather than botanical considerations.

Watt [1907] divided the genus into three main groups on :

(*a*) Position and shape of bracteoles, and whether united or free at the base.

(*b*) Presence or absence of extra-floral nectaries on the base of either the calyx tube or the bracteoles.

(*c*) Nature of lint and fuzz.

None of these characters is critical, since (*a*) though Asiatic cottons always have bracteoles more or less united at the base, in the American group both united and free bracteoles are found, (*b*) though Asiatic cottons very rarely have extra-floral nectaries on the base of the bracteoles, in the American group some types have such nectaries and others have not, and (*c*) fuzzy, tufted, and naked seeded types occur in all species in both Asiatic and American groups.

Watt described the Asiatic cultivated cottons as "fuzzy seeded cottons with united bracteoles". He made four species (*G. arboreum*, *G. Nanking*, *G. obtusifolium* and *G. herbaceum*) divided primarily on the shape of the leaves, and secondarily on the number of nectaries on the leaf nerves. He sub-divided the four species into a number of varieties on the character of their bracteoles, colour of their flowers and size of their bolls.

Leake and Ram Prasad [1914] classified the types used in their genetic experiments. Their main contribution was to emphasise the importance of branching habit, which they considered had not been adequately treated by Gammie or Watt.

Zaitzev [1928] was the first author to study the classification of *Gossypium* with the advantage of knowledge of the chromosome numbers of the Old World and New World groups, and his separation of them is consequently better than was that of any of his predecessors. Watt was especially at fault in attempting to explain the occurrence of combinations of characters not expected according to his system, as the result of hybridisation between species now known to cross only with extreme difficulty and to give completely sterile hybrids. Zaitzev made two groups of Old World cottons and two of New World cottons and gave a detailed comparison of their characters as illustrating the "law of homologous series". To him belongs the credit of first adequately determining the main grouping of the Asiatic cottons.

Harland [1928] in a short but important paper discussed Watt's [1926 ; 1927] later contributions to the taxonomy of *Gossypium* in the light of the results of modern genetic and cytological work and indicated the principles of a classification which should be acceptable to both geneticists and taxonomists. In his more detailed monograph Harland [1932] disagreed with Zaitzev's grouping of the New World cottons, and made three groups instead of Zaitzev's two, by sub-dividing Zaitzev's central American group into the Upland and Bourbon types.

Zaitzev excluded all wild species from the genus. Harland included most of the wild species given by Watt. He excluded *G. Kirkii* and *G. drynarioides* and included *G. trilobum* (*G. lanceaeforme*, or *Thurberia thespesioides*). Skovsted [1935] gave further reasons for including *G. trilobum* and added *G. aridum* (*Erioxylum aridum*). He also suggested that some species of certain other genera will be found to belong to *Gossypium*, notably *Cienfugosia triphylla*.

Chevalier [1933, 1935, 1936] has elucidated the status of *Gossypium* in Africa, especially in the French colonies south of the Sahara. In the first two papers [Chevalier, 1933 ; 1935], he discussed the taxonomy and distribution of *Gossypium anomalum* (*Cienfugosia anomala*) and concluded that it rightly belongs to *Gossypium* and not *Cienfugosia*. More recently he [Chevalier, 1936] has discussed the classification of the indigenous cottons of Africa in the light of a draft of this paper, and has given a valuable account of the African forms of *G. herbaceum* var. *frutescens* and intermediates between var. *frutescens* and var. *africanum*.

III. Classification

EVALUATION OF CHARACTERS

A source of much confusion in earlier work has been the occurrence in cotton of a group of simply inherited characters of large individual effect. When examined in the herbarium, specimens differing in one or two such characters are naturally and confidently classified as belonging to different species. A genetic study of the genus immediately revealed the deceptive nature of such differences and led to the discovery of specific distinctions based on differences in the hereditary make-up as a whole rather than on differences in particular characters. In devising a satisfactory classification for the species determined by genetic study, it is necessary to use only such characters as are indicative of fundamental differences in genetic composition, and the value of the more important characters for this purpose will be discussed in some detail.

Chromosome number

This character is of fundamental importance in the classification of the genus, but owing to the technical difficulty of its determination, it is of no practical value to the taxonomist. Two chromosome numbers occur, haploid 13 in Asiatic and African species, and in some wild American species, and haploid 26 in cultivated American species. For details see Skovsted [1935].

Plant habit

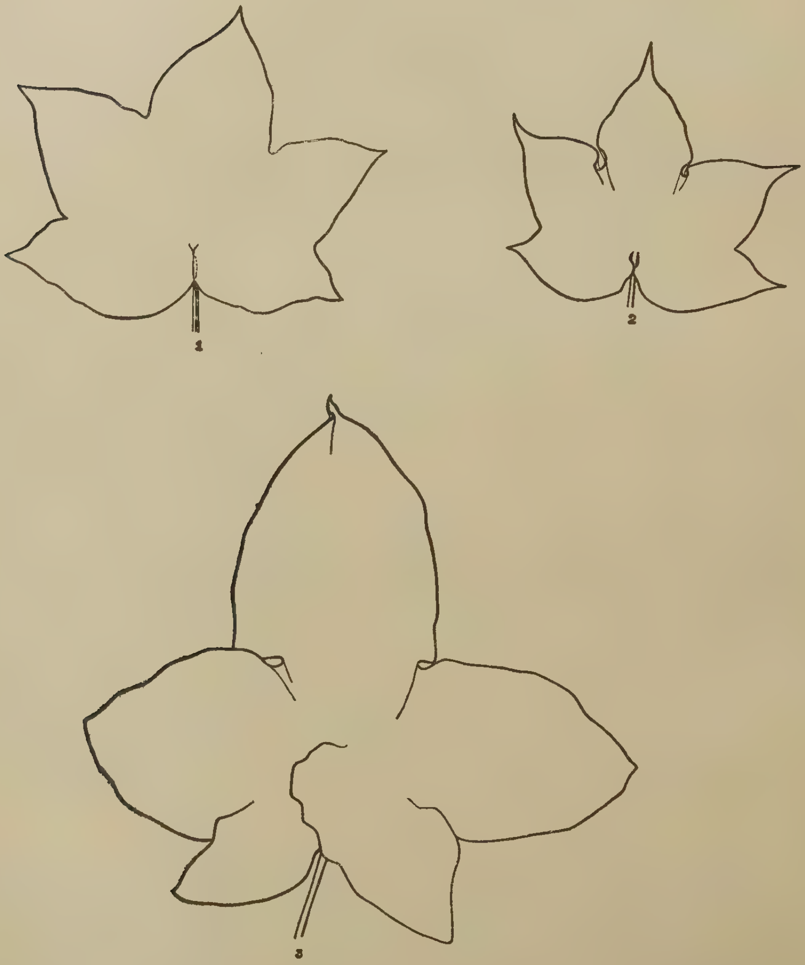
All cottons form two types of branches, monopodial vegetative branches, and sympodial fruiting branches. Plant habit depends on the relative proportion between the two and on the arrangement of the monopodial branches. It is of considerable taxonomic value, as the recent evolutionary trend throughout the cultivated sections of the genus appears to have been from the perennial, predominantly monopodial habit to small early-flowering, early-maturing annual, predominantly sympodial bushes.

This evolutionary trend appears to have developed subsequently to the differentiation of species, and consequently homologous series of habit types are to be found in most species.

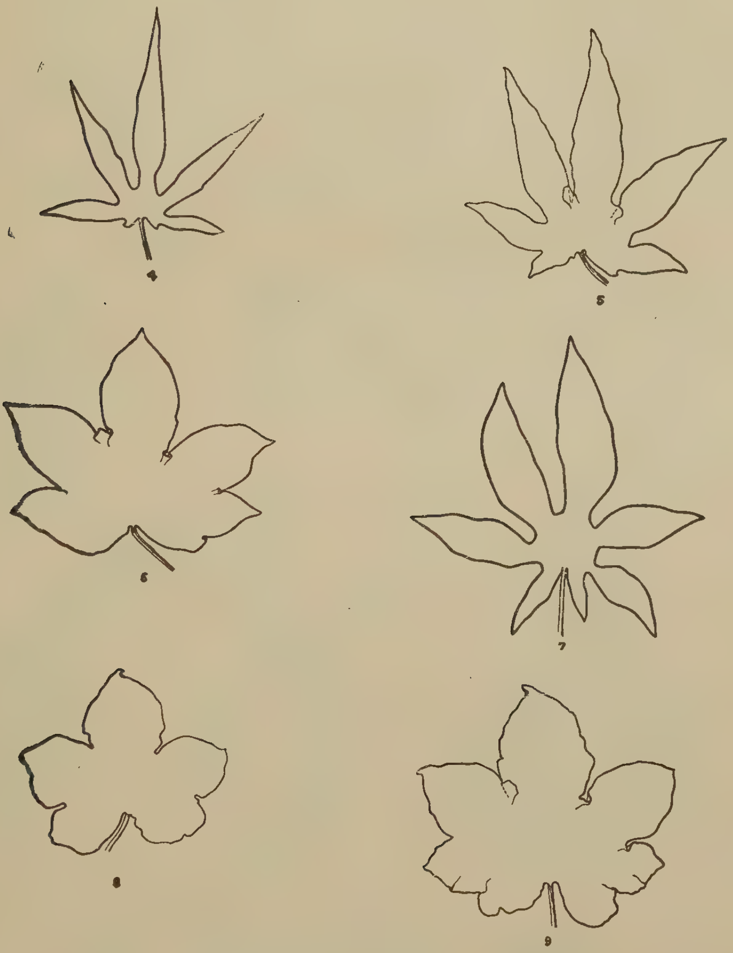
Shape of foliar organs

The leaf of *Gossypium* is—except in a few wild species—palmately divided more or less deeply into 3-7 lobes. The extent of the division varies from less than half-cut to deeply laciniated. It has been shown [Hutchinson, 1934] that the forms half-cut (broad), three-quarters-cut (' narrow '), and laciniated are, in the Asiatic cottons, simply inherited as a Mendelian multiple allelomorph series. It was also found [Hutchinson, *loc. cit.*] that neither the three-quarters-cut nor the laciniated form was really stable, both giving rise by mutation to the half-cut form. A similar series was demonstrated by Harland [1932] in New World cottons, involving ' Superokra ' or ' Willow leaf ' in which the lateral lobes of all leaves except the first three or four are lost ; ' okra leaf ' (the *G. Schottii* of Watt), which is a laciniated form ; and the normal half-cut palmatifid leaf of *G. hirsutum* or *G. religiosum*. Such differences, though of considerable importance to morphology, cannot be regarded as of taxonomic value, and their elimination removes the most serious anomalies from the older classifications. As an example may be quoted the distinction made by Watt between *G. arboreum* and *G. Nanking*. It is possible to select from commercial crops, plants of *G. arboreum* var. *neglectum* which, if the leaf shape difference is regarded as a valid specific distinction, will give progenies consisting of seventy-five per cent *G. arboreum* var. *neglectum*, and twenty-five per cent *G. Nanking*.

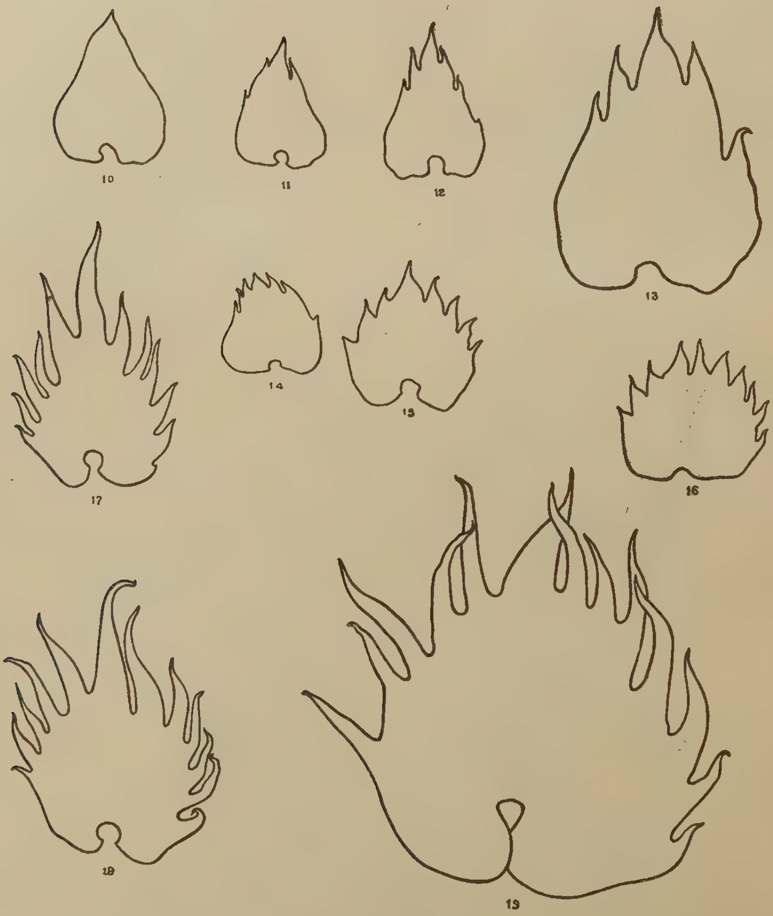
There is no correlation between the degree of laciniation of the leaf and the shape of other organs of foliar origin. There is in Asiatic cottons, however, a strong correlation between the shape of the leaf-lobe, the shape of bracteoles and bolls, the type of dentation of the bracteoles, and the type of surface (whether pitted or smooth) of the bolls. In Figs. 1—28 are given line drawings of leaves, bracteoles and bolls of typical forms. While in any one character some overlapping between species may be observed, the group as a whole exhibits a series of correlated forms which are the most valuable criteria for the separation of the Asiatic from the American species, and of the Asiatic species from each other.



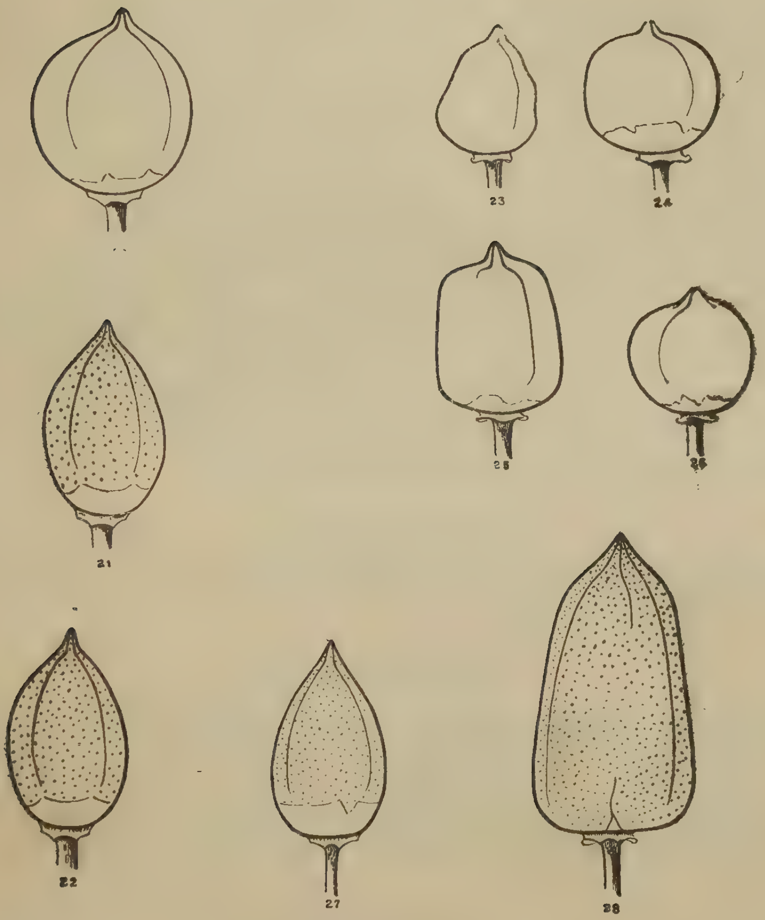
Figs. 1-3. Leaf outlines : New World cultivated species ($\times \frac{1}{4}$ nat. size) : 1. *G. hirsutum* L. 2. *G. religiosum* L. 3. *G. barbadense* L.



Figs. 4-9. Leaf outlines. Old World cultivated species (× ¼ nat. size): 4. *G. arboreum* L. var. *neglectum* Watt. 5. and 6. *G. arboreum* L. vars. *typicum* H. & G. and *neglectum* Watt. 7. *G. arboreum* L. var. *cernuum* H. & G. 8. *G. herbaceum* L. var. *typicum* H. & G. 9. *G. herbaceum* L. var. *frutescens* Delile.



Figs. 10-19. **Bracteole outlines (× $\frac{2}{3}$ nat. size) :**
10, 11, 12. *G. arboreum* L. vars. *typicum* H. & G. and *neglectum* Watt. 13. *G. arboreum* L. var. *cernuum* H. & G. 14, 15. *G. herbaceum* L. var. *typicum* H. & G. 16. *G. herbaceum* L. var. *frutescens* Delile. 17. *G. hirsutum* L. 18. *G. religiosum* L. 19. *G. barbadense* L.



Figs. 20-28. Boll outlines (× ⅔ nat. size):
20. *G. hirsutum* L. 21. *G. religiosum* L. 22. *G. barbadense* L. 23, 24. *G. herbaceum* L. var. *typicum* H. & G. 25, 26. *G. herbaceum* L. var. *frutescens* Delile. 27. *G. arboreum* L. vars. *typicum* H. & G. and *neglectum* Watt. 28. *G. arboreum* L. var. *cernuum* H. & G.

Anthocyanin pigment

Gammie and Watt confine *G. arboreum* to the type having red leaves and red flowers, though there is no mention of this character in Linnaeus's description. The difference between red and green types is inherited in a simple Mendelian manner, and is not correlated with any other character. It cannot, therefore, be regarded as of taxonomic value. In Asiatic cottons smaller differences in anthocyanin pigmentation are associated with specific differences, and exhibit the complex inheritance of characters controlled by a large number of hereditary factors. The most important of these characters is the presence in *G. arboreum* and absence in *G. herbaceum* of stem pigment and the red spot at the base of the leaf lamina. While this distinction is general, it is not absolute, owing to the interference of the simply inherited anthocyanin multiple allelomorph series [Hutchinson, 1932]. In *G. arboreum*, r^g types lack anthocyanin completely, and in *G. herbaceum* the heavily pigmented R^l and R types occur. Genetic analysis shows that these types carry the minor factor groups typical of their species and are, therefore, not strictly speaking exceptions, but they nevertheless make it impossible to use the character for diagnostic purposes.

Extra-floral nectaries

The distribution of this group of characters, which has been used by Watt, is subject to too many exceptions to be of value.

Nature of lint and fuzz

Watt based the primary divisions of the genus on this character. While the presence or absence of lint is of value in the separation of the wild lintless cottons from the cultivated species and their close relatives, the presence or absence of fuzz is of no use in classifying the cultivated cottons, as both fuzzy and naked seeded types occur in all species [Harland, 1932 ; Hutchinson, 1935].

KEY TO SPECIES

(*a*) Seed hairs short (less than ¼-in.), not arranged in two coats, removed from the seed only with great difficulty. Habit monopodial, with many vegetative branches. Stems thin and wand-like, almost glabrous. Leaves palmatifid. Bracteoles free, tapering to the base, not cordate (*b*).

  (*b*) Leaves deeply cut into 5-7 lanceolate lobes. Bracteoles linear lanceolate, usually deeply cut into three or four long, almost parallel teeth, sometimes entire. Bolls about twice as long as broad, tapering, giving a triangular outline, dotted with small glandular pustules, opening widely when ripe.

1. G. anomalum

TYPICAL HABIT FORMS IN *Gossypium arboreum* L.

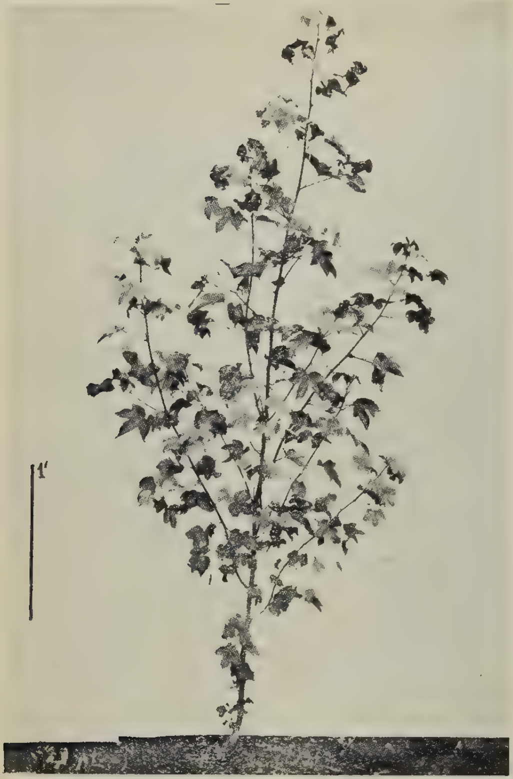


FIG. 1. *G. arboreum* L. var. *typicum* H. & G. (1056)

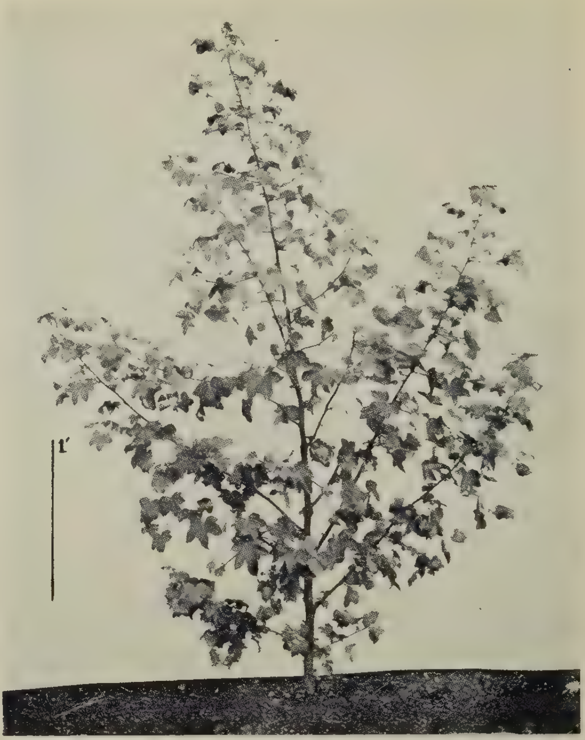


FIG. 2. *G. arboreum* L. var. *neglectum* Watt (Karunganni)

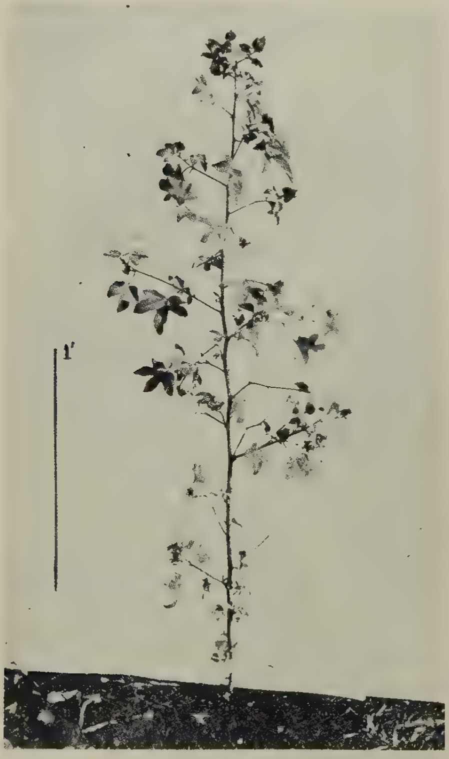


FIG. 3. *G. arboreum* L. var. *neglectum* Watt (Malvi 9)

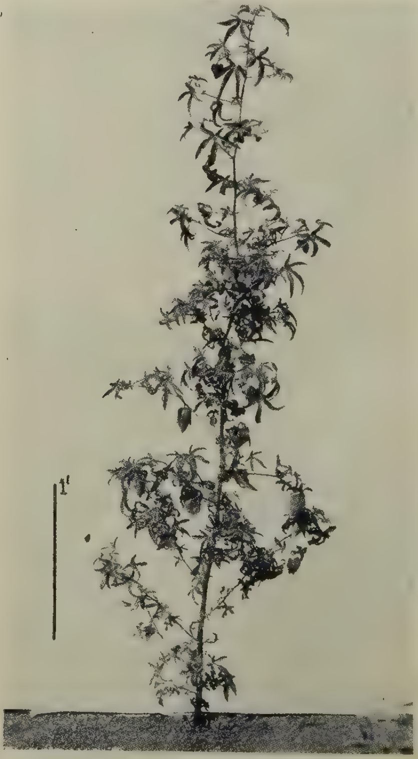


FIG. 4. *G. arboreum* L. var. *cernuum* H. & G. (Garo hills)

(*b*) Leaves cut less than half-way, into three to five rounded lobes which are deeply constricted at the base. Bracteoles only slightly longer than broad, tapering to a pronounced claw at the base, rounded at the apex, deeply cut into about ten long radiating teeth. Bolls round, smooth, prominently dotted with oil glands, which are below the surface; hardly opening when ripe.

2. *G. Stocksii*

(*a*) Seed hairs either divided into two coats, lint and fuzz, or consisting of lint only (*i.e.*, hairs ½-in. long or more) and usually easily separated from the seed. Leaves palmatifid. Bracteoles cordate, often united at the base, never clawed (*b*).

(*b*) Bracteoles triangular, longer than broad, entire or with three or four coarse teeth, closely investing the bud, flower and boll, occasionally spreading away from the apex of the boll (Figs. 10-13). Bolls tapering profusely pitted, with prominent oil glands in the pits (Figs. 27 and 28) (*c*).

(*c*) Habit monopodial with many vegetative branches, growing into a bushy shrub (Plate XII, fig. 1). Bolls 1-in. to 1¾ in. long, not more than eleven seeds per loculus (Fig. 27).

3*a*. *G. arboreum* var. *typicum*

(*c*) Habit sympodial, with few or no vegetative branches, growing into a small shrub, not bushy, 2 ft. to 6 ft. high (*d*).

(*d*) Bolls 1-in. to 1¾-in. long, not more than eleven seeds per loculus (Fig. 27 and Plate XII, fig. 3).

3*b*. var. *neglectum*

(*d*) Bolls 2-in. or more long, with thirteen to seventeen seeds per loculus (Fig. 28 and Plate XII, fig. 4).

3*c*. var. *cernuum*

(*b*) Bracteoles rounded or broadly triangular, broader than long, with margin divided into a number of triangular teeth, flaring widely from the base of the bud, flower and boll (Figs. 14-16). Bolls rounded, or with prominent shoulders, boll surface smooth, or very shallowly dented, with few or no oil glands (Figs. 23-26) (*c*).

(*c*) Habit sympodial, without or with very few spreading vegetative branches (Plate XIII, fig. 1). Petioles, pedicels and leaves hairy or sparsely hairy. Stems almost glabrous below. Leaves large, leathery, usually flat and only slightly constricted at the base of the lobe (Fig. 8). Bolls 1-in. to 1¾-in. long, not more than eleven seeds per loculus (Figs. 23 and 24).

4a. G. herbaceum var. *typicum*

(*c*) Habit sympodial or monopodial with 0 to 10 ascending vegetative branches (Plate XIII, fig. 2). Young shoots woolly. Stems, petioles, pedicels and leaves intensely hairy. Leaves large, thick, rumpled, and only slightly constricted at the base of the lobe (Fig. 9). Bolls 1-in. to 1¾-in. long, not more than eleven seeds per loculus (Figs. 25 and 26).

4b. var. *frutescens*

(*c*) Habit monopodial, with many ascending vegetative branches. Leaves small, thin, flat, and deeply constricted at the base of the lobes. Stems, petioles, pedicels and leaves almost glabrous. Bolls ¾-in. long or less, usually five to seven seeds per loculus.

4c. var. *africanum*

(*b*) Bracteoles gashed into a varying number of long acuminate teeth, closely investing the bud, flower and boll (Figs. 17-19) (*c*).

(*c*) Habit varying from sympodial to monopodial (Plate XIV, fig. 1). Leaf-lobes ovate-oblong, usually overlapping at the base, causing the sinuses to be thrown up in folds (Fig. 3). Corolla not widely expanding, forming a narrow cup. Bolls long, tapering, shiny, dark green, profusely pitted, with oil glands at the base of the pits (Fig. 22).

5. G. barbadense

(*c*) Habit moderately to highly monopodial, with a number of large vegetative branches (Plate XIII, fig. 3). Leaves occasionally entire, rarely palmatisect. Leaf-lobes broadly ovate, almost triangular, divergent, not constricted at the base, not, or only very slightly, overlapping (Fig. 2). Corolla not widely expanding, forming a narrow cup. Bolls rounded or tapering, pale green, surface smooth, with oil glands sunk below it (Fig. 21).

6. G. religiosum

TYPICAL HABIT FORMS IN *Gossypium herbaceum* L. & *G. religiosum* L.

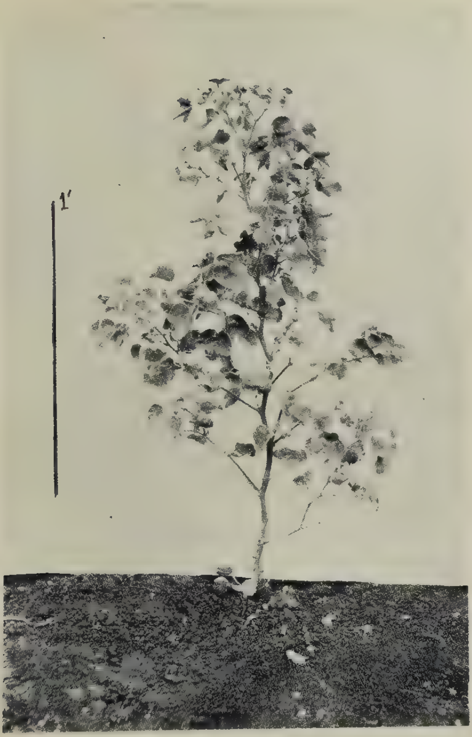


FIG. 1. *G. herbaceum* L. var. *typicum* H. & G. (H 14)

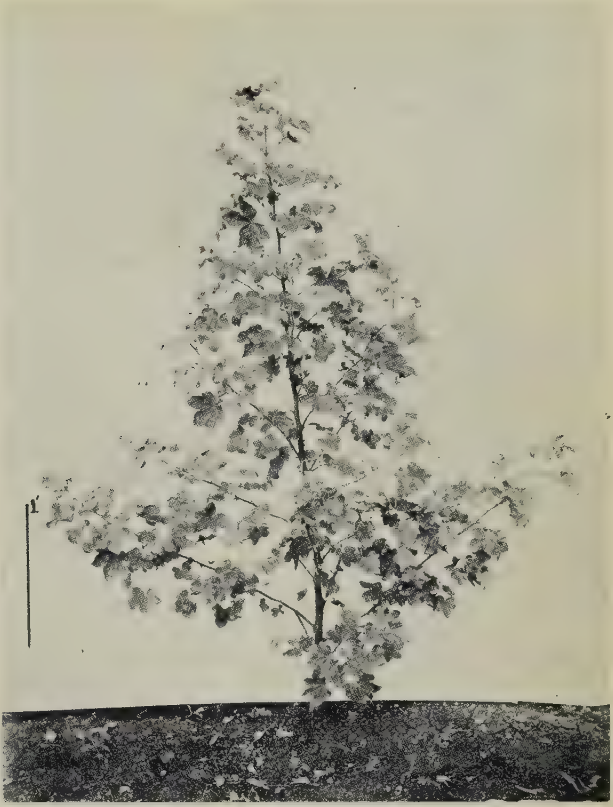


FIG. 2. *G. herbaceum* L. var. *frutescens* Delile (Broach 9)

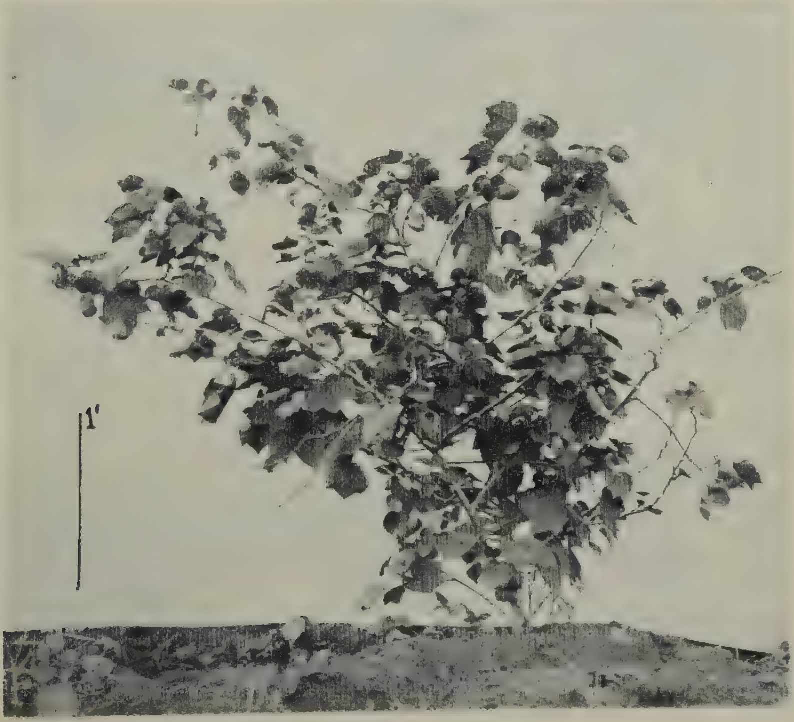


FIG. 3. *G. religiosum* L. (Bourbon)

(c) Habit sympodial, with no or very few vegetative branches (Plate XIV, fig. 2). Leaves occasionally palmatisect. Leaf-lobes divergent, broadly triangular, not constricted at the base, not overlapping (Fig. 1). Corolla widely expanding. Bolls rounded, often large, pale green, surface smooth, oil glands few and sunk beneath the boll surface (Fig. 20).

7. G. hirsutum

DESCRIPTION OF SPECIES

1. Gossypium anomalum *Wawra and Peyr., in Sert. Benguel 21 (1860)*

Haploid chromosome number 13. Shrub 5 ft.-8 ft. high (when grown under experimental conditions), monopodial in habit, with many vegetative branches. Stems thin and weak, almost glabrous. Leaves large (about 4-in. long), palmatifid, almost palmatisect, deeply divided into five to seven lanceolate lobes. Bracteoles free at the base, linear lanceolate, usually deeply cut into three or four almost parallel teeth, flaring away from the bud, flower and boll. Bolls much longer than broad, strongly tapering so as to be almost triangular in longitudinal section, dotted with small glandular pustules, opening when ripe and allowing the seeds to fall out. Seeds long and thin, with a single coat of fine brown hairs.

Distribution.—Angola, French Sudan and Somaliland. The types (*Wawra* 262 and 285) were collected in Benguela, Angola.

2. Gossypium Stocksii *Mast. in Hook. f. Fl. Br. Ind. 1 : 346 (1874)*

Haploid chromosome number 13. Shrub 2 ft.-4 ft. high, monopodial in habit, with many vegetative branches. Stems thin and weak, almost glabrous. Leaves small (about 1-in. long), shallowly palmatifid, half-cut into three to five rounded, deeply constricted lobes. Bracteoles free at the base, clawed, only slightly longer than broad, rounded, deeply cut into about ten radiating teeth, flaring away from the bud, flower and boll. Bolls small, round, smooth, prominently dotted with oil glands, hardly opening when ripe, retaining the seeds within. Seeds small, not much longer than broad, with a single coat of rusty red hairs.

Distribution.—Sind and South East Arabia.

3. Gossypium arboreum *L. Sp. Pl. 693, 1753, sec. Watt*

The type is the specimen in Linné's herbarium labelled "3. *G. arboreum*" in Linné's handwriting, figured by Watt [1907, pl. 7 c]. The specimen in the Linnean herbarium labelled "5. *barbadense ?*" is the broad-leaved form of the species, probably the *Rozi* of Western India. It is figured by Watt [1907, pl. 19A].

Haploid chromosome number 13. Shrub 2 ft.-10 ft. high. Stems weak and wand-like, intensely hairy to almost glabrous. Leaves palmatifid to palmatisect, three to seven lobed, usually cordate at the base, red or dark green, with or without nectaries on the main nerves ; lobes divergent, never overlapping, ovate-oblong or curvilinear, not markedly acuminate, slightly constricted at the base, often with small accessory lobes in the sinuses between the main lobes (Figs. 4-7). Bracteoles triangular, longer than broad, slightly to strongly cordate, entire or with three or four coarse teeth, closely investing the bud and flower, and usually the boll. (Occasionally types are found in which the bracteoles spread somewhat from the apex of the boll, but the base is always closely invested) (Figs. 10-13). Flowers red, yellow or white, with or without a red spot at the base of the petal. Bolls tapering, profusely pitted, with prominent oil glands in the pits, red or green, opening widely when ripe (Figs. 27 and 28). Seeds small. Lint white, grey or brown. Fuzz green, grey or white (brown in brown linted forms), uniformly distributed over the seed, or confined to a tuft at each end.

(*a*) var. *typicum*, Hutchinson and Ghose. Includes Watt's [1907 and 1926] *G. obtusifolium* Roxb., *G. Nanking* Meyen vars. *Nadam, Rozi* and *soudanensis* Watt, *G. Simpsoni* Watt, *G. soudanense* Watt, *G. abyssinicum* Watt, and *G. Nanking* Meyen var. *canescens* Watt (in part) ; Gammie's [1907] *G. obtusifolium* Roxb. and *G. intermedium* Todaro ; and *G. herbaceum* L. var. *Perrieri* Hochreutiner [1926].

Habit monopodial, with many vegetative branches, growing into a bushy shrub (Plate XII, fig. 1). Bracteoles not continuing to grow, or growing only slightly, after the flower has faded (Figs. 10-12). Bolls 1-in. to 1¾-in. long, not more than eleven seeds per loculus (Fig. 27). Four forms may be distinguished, chiefly on geographical grounds.

I. Forma *bengalensis*, Hutchinson and Ghose. We include in this *G. arboreum* L. (Watt) (in part), and *G. intermedium* Todaro of Gammie. Erect pyramidal shrubs, with many ascending vegetative branches. Stems, petioles, and leaves hairy. Leaves often three-quarters cut. Bracteoles small, not continuing to grow after the flower has faded, considerably shorter than the boll.

Distribution.—Assam, Bengal, Gangetic plain and Central India.

II. Forma *burmanica*, Hutchinson and Ghose. We include *G. arboreum* L. (Watt) (in part), *G. arboreum* L. var. *Nadam* Watt (in part), *G. obtusifolium* Roxb. (Watt) (in part), and *G. obtusifolium* Roxb. (Gammie).

Similar to forma *bengalensis*, but bracteoles rather large, broad, and deeply cordate, sometimes growing slightly after the flower has faded, almost as long as the boll.

Distribution.—Burma, Malaya, Dutch East Indies, Siam, French China and the Philippines.

III. Forma *indica*, Hutchinson and Ghose. We include *G. obtusifolium* Roxb. (of Watt and Gammie), except Burmese and farther eastern types, and *G. Nanking* Meyen, vars. *Rozi*, and *Nadam* Watt.

Round-headed shrubs. Wild types sub-scandent, cultivated types erect. Leaves never more than two-thirds cut. Stems, petioles and leaves moderately hairy to almost glabrous. In many types, the stems become glabrous as they become mature. The wild types are usually almost completely glabrous, and have very short lint and long fuzz. Bracteoles small, not continuing to grow after the flower has faded, considerably shorter than the bolls.

Distribution.—Peninsular India south of the Tapti river, Kathiawar and Gujerat, and in Ceylon.

IV. Forma *soudanensis*, Hutchinson and Ghose. Here we include *G. Nanking* Meyen var. *soudanensis* Watt, and var. *canescens* Watt (in part), *G. Simpsoni* Watt, *G. soudanense* Watt and *G. abyssinicum* Watt, and *G. herbaceum* L. var. *Perrieri* Hochreutiner [1926].

Similar to forma *indica*.

Distribution.—Abyssinia and the Sudan, northern Nigeria, Angola, Zambesia, southern Arabia and Madagascar.

(*b*) var. *neglectum* Watt [1907] ampl. Hutchinson and Ghose. We here include *G. arboreum* L. vars. *sanguinea* and *rosea* Watt ; *G. Nanking* Meyen and its vars. *himalayana*, *rubicunda*, *bani*, *canescens* Watt (in part), and *japanense* Watt, and *G. anomalum* Watt of Watt [1907, 1926, 1927] ; also *G. sanguineum* Hassk., *G. indicum* Lamk., and *G. neglectum* Todaro and their varieties of Gammie [1907].

Habit sympodial with few or no vegetative branches, growing into a small shrub, not bushy (Plate XII, fig. 3). Stems petioles and leaves usually hairy. Bracteoles not continuing to grow, or growing only slightly, after the flower has faded (Figs. 10-12). Bolls 1-in. to 1¾-in. long, not more than eleven seeds per loculus (Fig. 27).

Four forms may be distinguished, chiefly on geographical grounds, corresponding to the four forms of var. *typicum*.

I. Forma *bengalensis*, Hutchinson and Ghose. We include here parts of *G. arboreum* L. vars. *sanguinea*, *neglecta*, and *rosea* Watt, and *G. Nanking* Meyen vars. *himalayana*, *rubicunda*, and *bani* Watt [1907], and Gammie's *G. sanguineum* Hassk. and *G. neglectum* Todaro and their varieties, and *G. indicum* Lamk. (in part), and its var. *Mollisoni* Gammie.

Very variable in simply inherited morphological characters, *e.g.*, leaf-shape flower-colour, and anthocyanin pigmentation. Stems, petioles and leaves hairy. Bracteoles small, not continuing to grow, or only growing slightly after the flower has faded.

Distribution.—Assam, Bengal, Gangetic plain, Punjab, North-West Frontier Province, Rajputana, Central India, Kathiawar, Gujerat, Khandesh, and parts of the Central Provinces. A few small areas, mostly of recent introduction, in Madras.

II. Forma *burmanica*, Hutchinson and Ghose. We include parts of *G. arboreum* L. vars. *sanguinea*, *neglecta*, and *rosea* Watt, and *G. Nanking* Meyen and its var. *bani* Watt [1907], and *G. Nanking* Meyen var. *japanense* Watt and *G. anomalum* Watt [1926, 1927] and parts of *G. neglectum* Todaro, of Gammie [1907].

Very variable in simply inherited morphological characters, *e.g.*, leaf-shape, flower-colour, and anthocyanin pigmentation. Stems, petioles and leaves hairy (in Burmese types), or almost glabrous (in many Chinese types). Bracteoles usually rather large, broad, and frequently growing slightly after the flower has faded.

Distribution.—Burma, Malaya, Dutch East Indies, Philippines, Siam, French China, China, Formosa and Japan.

III. Forma *indica*, Hutchinson and Ghose. We include *G. arboreum* L. var. *bani* Watt [1907] (in part), and *G. indicum* Lamk. of Gammie [1907] (in part).

Uniform in most morphological characters, almost always having yellow flowers, and broad leaves (half to two-thirds cut). Stems, petioles and leaves hairy. Bracteoles small, not continuing to grow after the flower has faded.

Distribution.—Peninsular India south of the Tapti river, Ceylon.

IV. Forma *soudanensis*, Hutchinson and Ghose. We include *G. arboreum* L. vars. *sanguinea*, *neglecta* and *rosea* Watt [1907] (in part), and *G. Nanking* Meyen var. *canescens* Watt [1926].

Variable in simply inherited morphological characters, *e.g.*, leaf-shape, flower-colour and anthocyanin pigmentation. Stems, petioles and leaves hairy, canescent, or almost glabrous.

Distribution.—Abyssinia, Anglo-Egyptian and French Sudan, Northern Nigeria and Gold Coast, Dahomey and Senegal.

(*c*) var. *cernuum* Hutchinson and Ghose, (comb. nov.). We include *G. arboreum* L. var. *assamica* Watt [1907]; *G. cernuum* Todaro (partly); *G. cernuum* Todaro var. *silhetensis* Gammie [1907] (partly).

Habit sympodial, with few or no vegetative branches, growing into a small shrub, not bushy (Plate XII, fig. 4). Stems, petioles and leaves almost glabrous. Bracteoles continuing to grow after the flower has faded, finally growing about as long as the boll (Fig. 13). Bolls not less than $1\frac{3}{4}$-in., usually 2-in. or more long, with thirteen to seventeen seeds per loculus (Fig. 28).

Distribution.—Confined to Assam and East Bengal.

4. Gossypium herbaceum *L. Sp. Pl. 693, 1753, sec. Watt [1907]*

The type is the specimen in Linné's herbarium labelled " 1. *herbaceum* ex Hort. Upsala," figured by Watt [1907, Pl. 24A]. Watt's discussion [1907, p. 160 *et sec.*] of the nomenclature is an excellent summary of the reasons for confining the name *herbaceum* to what he calls the 'Levantine species' and his definition of the type of the species is accepted here.

Haploid chromosome number 13. Shrub 2 ft.-8 ft. high. Stems thick and rigid. Leaves palmatifid, three to seven lobed, usually cordate at the base, red or light green, with nectaries on the main nerves ; lobes divergent, ovate rotund to rounded, usually somewhat constricted at the base, without accessory lobes in the sinuses between the main lobes (Figs. 8 and 9). Bracteoles rounded or broadly triangular, broader than long, slightly to strongly cordate, margin divided into six to eight broadly triangular teeth, beginning to flare away from the base of the bud when it is about $\frac{1}{4}$-in. long, and flaring widely from the flower and boll (Figs. 14-16). Flowers yellow, rarely white, with, or rarely without, a red spot at the base of the petal. Bolls 1-in. to $1\frac{3}{4}$-in. long, rounded, or with prominent shoulders, beaked, surface smooth, or very shallowly dented, with few or no oil glands, red or green, in many types remaining almost completely closed when ripe, not more than eleven seeds per loculus (Figs. 23-26). Lint white, grey, or red-brown. Fuzz grey or white (brown in brown linted forms) uniformly distributed over the seed, in rare types completely absent.

(*a*) var. *typicum,* Hutchinson and Ghose. Under this we include only *G. herbaceum* L. as interpreted by Watt [1907].

Habit sympodial, without or with very few vegetative branches (Plate XIII, fig.1). Stems, petioles, pedicels and leaves sparsely hairy to almost glabrous. Leaves leathery, usually flat, with divergent lobes only slightly constricted at the base with open sinuses between them (Fig. 8). Bolls usually rounded, rarely with prominent shoulders, usually remaining closed, or opening only slightly when ripe (Figs. 23 and 24). Seeds large or small, usually densely fuzzy.

Distribution.—Iran, Afghanistan, Russian Turkestan, Baluchistan, Iraq, Asia Minor, South Eastern Europe, North Egypt and Libya.

(*b*) var. *frutescens* Delile, Fl. Ægypt., Illustr. p. 69 t II [1813]. Under this we include *G. Wightianum* Todaro [1877], *G. obtusifolium* Roxb. var. *Wightiana* Watt [1907] and *G. herbaceum* L. and its varieties, Gammie [1907]. The specimen in the Linnean herbarium labelled ' 4. *G. hirsutum*, Suratt ' belongs here.

Habit monopodial, with four to ten strong vegetative branches, sympodial in south Indian types (Plate XIII, fig. 2). Stems, petioles, pedicels, and leaves densely hairy. Leaves thick, strongly rumpled, with lobes slightly constricted at the base, and with sinuses overlapping or thrown up in folds (Fig. 9). Bolls almost parallel sided with prominent shoulders, opening widely when ripe, or rounded, opening only slightly when ripe (Figs. 25 and 26). Seeds small, usually fuzzy, in rare types entirely free from fuzz, and carrying only a much reduced coating of lint.

Distribution.—Kathiawar, Gujerat, and the Bombay and Madras Presidencies in India; the region between the Sahara and the forests of tropical Africa from Nubia to Senegal, Zambesia and the districts of Shire and Lake Nyasa (Nyasaland), in Africa.

(*c*) var. *africanum* Hutchinson and Ghose (comb. nov.). The type is Mrs. Lugard 198 from Kwebe Hills, Ngamiland, described by Watt [1926] as *G. africanum*. We include *G. obtusifolium* Roxb. var. *africana* Watt [1907] and *G. africanum* var. *bracteatum* Watt and *G. transvaalense* Watt [1926].

Habit monopodial, with many vegetative branches, forming a bushy shrub. Stems thin, weak and wand-like. Stems, petioles and pedicels slightly hairy when young, but becoming almost completely glabrous at maturity. Leaves, thin, flat; leaf-lobes rounded, deeply constricted at the base, with open sinuses between the lobes. Bolls small, less than ¾-in. long, rounded, smooth, green, opening widely when ripe. Seeds small with a thin white or grey fuzz. Lint ashy grey, very sparse.

Distribution.—Chiefly in the Bushveld regions of South Africa from Bechuanaland to Moçambique.

Chevalier [1936] describes a series of intermediate types linking var. *frutescens* with var. *africanum*. Specimens in the Kew Herbarium from Bornu (West Africa) Kordofan (Sudan), and Madanda forest (British East Africa) assigned by Watt [1926] to *G. africanum* or *G. transvaalense* appear to be such intermediates.

5. Gossypium barbadense *L. Sp. Pl. 693 (1753).*

The type is Plukenet's specimen in the British Museum, figured by Watt [1907, Plate 46 A]. The sheet labelled '*barbadense?*' in the Linnean herbarium is not this species. Under this name we include *G. mustelinum* Meirs, *G. microcarpum* Todaro, *G. peruvianum* Cav., *G. vitifolium* Lamk., and *G. braziliense* Macf.

Haploid chromosome number 26. Shrubs or small trees, 3 ft.-15 ft. high; moderately to highly monopodial (Plate XIV, fig. 1). Stems strong, forming fair sized trunks in the larger types. Leaves palmatifid, rarely entire, usually three to five lobed, sinuses thrown up in folds; lobes ovate-oblong, strongly acuminate, very slightly constricted at the base (Fig. 3). Corolla not widely expanding, forming a narrow cup. Bracteoles triangular, cordate, gashed into a number of acuminate teeth, closely investing the bud, flower and boll (fig. 19). Bolls tapering, sharply pointed, with glands at the base of shallow crater-like depressions, shiny dark green (Fig. 22).

TYPICAL HABIT FORMS IN *Gossypium barbadense* L. & *G. hirsutum* L.

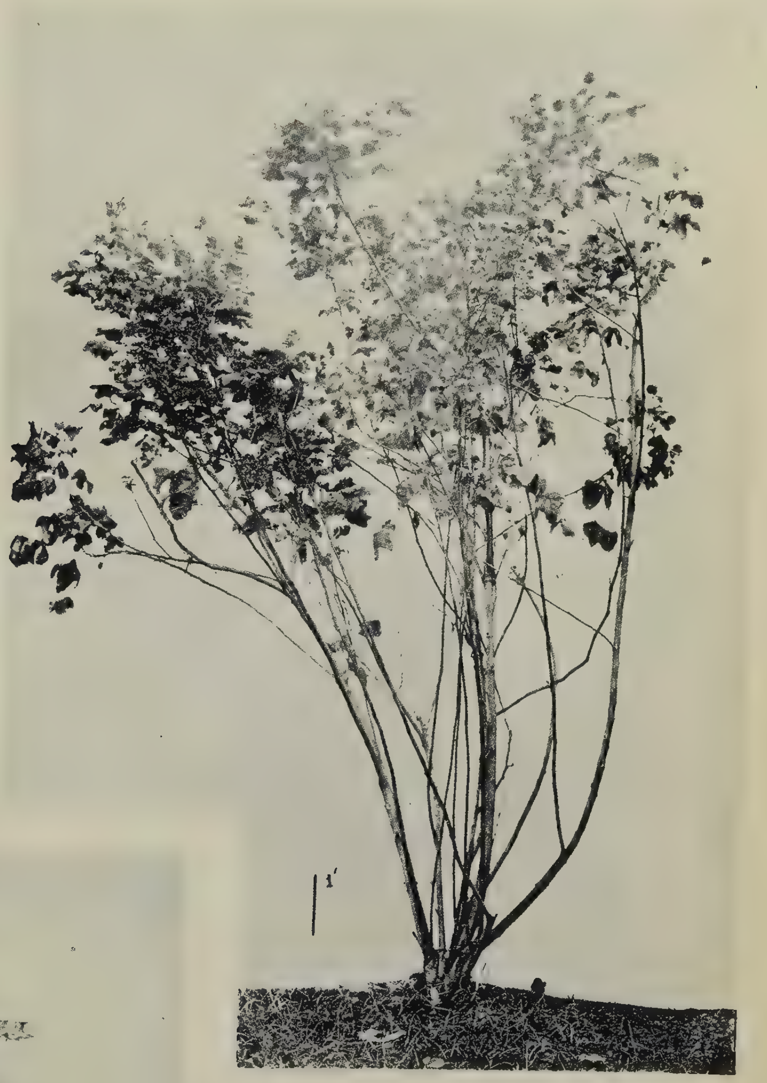


FIG. 1. *G. barbadense* L. (Kidney)

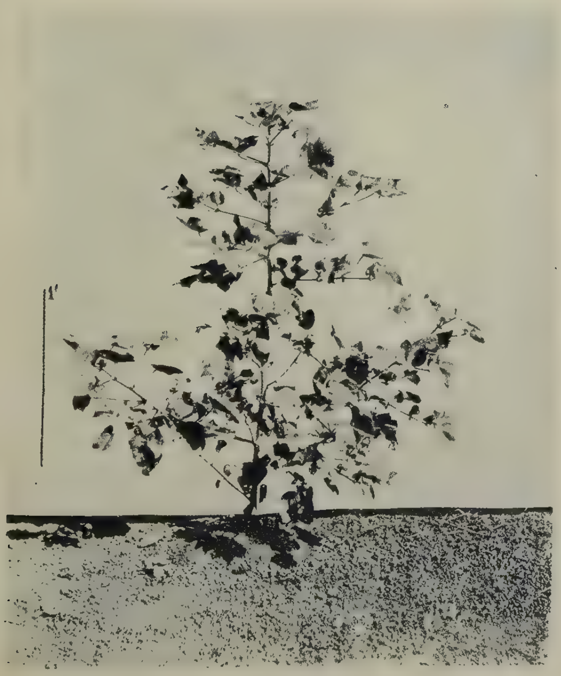


FIG. 2. *G. hirsutum* L. (Indore 1)

Distribution.—Indigenous in South America and the West Indies. Naturalised in many places in Asia and Africa, especially Egypt and Nigeria.

6. Gossypium religiosum *L. Syst., Ed. 12 : 642* (*1766-8*)

The type is the specimen in the Linnean herbarium labelled '*religiosum*' in Linné's handwriting, and is figured by Watt [1907, Pl. 32 A]. We include here *G. purpurascens* Poir. (the Bourbon group) of Harland [1932], and *G. purpurascens* Poir., *G. punctatum* Sch. et Thon., *G. Schottii* Watt, (in part), and *G. hirsutum* L. var. *religiosa* Watt [Watt, 1907].

Haploid chromosome number 26. Medium shrubs or small trees 4 ft.-15 ft. high with a number of large vegetative branches (Plate XIII, fig. 3). Stems strong, forming fair-sized trunks in the larger types. Leaves shallowly palmatifid, sometimes entire, especially on old plants, in rare types palmatisect, usually three sometimes five lobed ; lobes divergent, broadly ovate, almost triangular, acuminate, not constricted at the base, sinuses between lobes open, not, or only very slightly, overlapping (Fig. 2). Corolla not widely expanding, forming a narrow cup. Bracteoles triangular, cordate, gashed into a number of acuminate teeth, closely investing the bud, flower and boll (Fig. 18). Bolls rounded, boll surface smooth, green, with glands sunk below the surface (Fig. 21).

Distribution.—Indigenous in Central America, West Indies and parts of South America. Naturalised in many scattered areas in Asia and Africa.

7. Gossypium hirsutum *L. Sp. Pl. Ed. 2 : 975* (*1763*)

The type is Miller's specimen in the British Museum herbarium figured by Watt, [1907, Pl. 29 A]. We include *G. mexicanum* Todaro and *G. Schottii* (in part) Watt [1907]. The specimen labelled '*hirsutum*' in the Linnean herbarium is *G. herbaceum* var. *frutescens*.

Haploid chromosome number 26. Small shrubs 1 ft.-5 ft. high, sympodial, with few or no vegetative branches (Plate XIV, fig. 2). Stems strong. Leaves shallowly palmatifid, in rare types palmatisect, usually three rarely five lobed ; lobes divergent, broadly triangular, acuminate, not constricted at the base, sinuses open, not overlapping (Fig. 1). Corolla widely expanding. Bracteoles triangular, cordate, gashed into a number of acuminate teeth, closely investing the bud, flower and boll (Fig. 17). Bolls rounded, often large, boll surface smooth, green, glands few and sunk beneath the surface (Fig. 20).

Distribution.—Indigenous in Central and Southern North America. Naturalised in all cotton-growing areas of Asia and Africa.

IV. Discussion

The merging of *G. obtusifolium, G. indicum* and *G. Nanking* in *G. arboreum* follows Zaitzev and is undoubtedly in agreement with the botanical facts. *G. obtusifolium* was insufficiently described in the first place and has been a source of confusion ever since. In the classification given in Harland's [1932] monograph, *Nanking* is allowed varietal rank. Further consideration, and examination of Indian cottons in India has shown this to be untenable, as it cuts across both *G. arboreum* and its var. *neglectum.*

The distinction between *G. arboreum* var. *typicum* and var. *neglectum* may occasionally cause some difficulty. The habit of the plant is usually adequate to enable classification to be made with certainty, but intermediate types are to be expected, and their existence is not regarded as impairing the validity of the classification.

The distinguishing characteristics of *G. arboreum* var. *cernuum* may be emphasised. They are :—(1) Size of the boll and number of seeds per loculus, and (2) Continued growth of the bracteoles after the fading of the flower. The custom common in India of classifying as *G. arboreum* var. *cernuum* (or its synonym, *G. cernuum*) all coarse linted types of *G. arboreum* var. *neglectum* with laciniated leaves is botanically unjustifiable. Associated with the large boll is a characteristic elongation of all parts of foliar origin. Attention has been drawn by Hutchinson [1936] to the correlation existing between various organs of foliar origin in size and shape, and the present case appears to be another example of the same association. Leaves, petals, and bracteoles are all longer in var. *cernuum* than in var. *neglectum.*

It is of interest to observe that there is a parallelism in many characters between *G. anomalum* and *G. arboreum*, and between *G. Stocksii* and *G. herbaceum. G. anomalum* has leaves deeply cut into lanceolate lobes strongly reminiscent of those of narrow-leaved types of *G. arboreum*, bracteoles with few teeth, and tapering pointed bolls which open and allow the seed to drop out on ripening. *G. Stocksii* has leaves less than half cut into rounded lobes, rounded bracteoles with many teeth, and rounded bolls hardly opening when ripe.

Chevalier [1933] and Skovsted [1935] drew attention to *Cienfugosia triphylla* Engler from South-West Africa, and suggested that it should be transferred to *Gossypium* as *Gossypium triphyllum.* It is worth mentioning at this point, as it is probably related to *G. anomalum*, but no further discussion is possible in the absence of adequate material.

G. anomalum and *G. Stocksii* can be crossed with *G. herbaceum* and *G. arboreum* without serious difficulty. All hybrids with *G. Stocksii* have proved completely sterile. They are variable in vigour, hybrids with some types of *G. arboreum* var. *neglectum* being quite vigorous, while the only recorded hybrid with *G. herbaceum* var. *frutescens* was very feeble, and a hybrid with *G. anomalum* died at an early stage [Skovsted, 1935]. Hybrids between *G. anomalum*, and either *G. herbaceum* or *G. arboreum* are slightly fertile. Skovsted [1935] reports that back-cross seedlings died at an early stage. At Indore F_2s, F_3s, and back-crosses of both *G. anomalum* × *G. arboreum* var. *neglectum* and *G. anomalum* × *G. herbaceum* have been grown successfully, and have exhibited varying degrees of fertility.

All wild cottons are perennial, highly monopodial, much branched shrubs. The development of the forms of modern cultivation has proceeded through an evolutionary sequence which can be followed in all species which have been exploited by man.

The most primitive cultivated types are similar to the wild types in most characters, but have much superior lint, and generally tend to produce sympodial fruiting branches at an earlier stage. The sub-scandent habit common in wild types never persists in the cultivated forms. The resemblance between wild types and their nearest cultivated relatives is so close in the two cultivated Old World species that the older botanists usually described the truly wild, sub-scandant forms of *G. arboreum* found in hedgerows and on old ruins in many parts of India, as escapes from cultivation ; and in Africa, Chevalier [1936] has been able to describe a range of types uniting the truly wild *G. herbaceum* var. *africanum* of the South African bushveld with the cultivated forms of *G. herbaceum* var. *frutescens* of Zambesia and the French colonies between the Sahara and the Congo forests.

With the establishment of cultivation, the perennial types became subject to the influence of two new selective forces. The first was reduction in the length of the growing season. If cotton was to be grown in areas with long dry seasons, or cold winters, annual types were essential, and the Upland cottons of America (*G. hirsutum*), the *herbaceum* cottons of Africa and Central Asia, and the *neglectums* of northern India, China and Japan are the products of evolution under the selective action of this factor. Later, with the development of large scale agriculture, the annual habit became desirable on account of ease of cultivation, and better control of pests and diseases, and even in areas where the growth of perennials was possible, the development of annual types took place. Hence arose the annual types of *G. barbadense* (Sea Island and Egyptian), and the annual *neglectums* of South India.

The foundations of an understanding of the distribution of the indigenous cottons of Asia and Africa were laid by Zaitzev [1928]. He considered that *G. herbaceum*, and its varieties belonged properly to Africa, and *G. arboreum* and its varieties to Indo-China.

Chevalier [1936] has so far elucidated the status of *G. herbaceum* in Africa that it is now possible to trace with some confidence a sequence of forms from the truly wild *G. herbaceum* var. *africanum* to *G. herbaceum* var. *frutescens* of Africa and of western India, and finally to *G. herbaceum* var. *typicum* of Iran, Afghanistan and Turkestan. A secondary centre of variability occurs in var. *frutescens* in Gujerat, where Patel [1920] states that the three main types of the variety " will be found mixed and hybridised in every proportion in almost every part of the province". Southwards the variability decreases, and the moderately monopodial, branched, late types of Gujerat give place to sympodial, early types in the Bombay-Deccan and Madras. In northern Kathiawar and Cutch the earlier, less branched Wagad predominates, and in this form of var. *frutescens* the closed boll commonest in var. *typicum* first appears. A secondary centre of variability has been found by the Russian workers in var. *typicum* in northern Iran and Afghanistan [Chernyakovskaya, 1930].

In *G. arboreum* no such simple sequence is traceable, but the inter-relationships of the various forms of the species become clear when they are regarded as the results of the action of similar selective forces on a widely distributed primitive type. In the description of the species given above, var. *typicum* and var. *neglectum* have both been divided into the same set of four forms. This method of classification has been used in an attempt to separate the effects of geographical accident from the results of the action of common selective forces. The primary selective force concerned has been that in favour of the annual habit, and the results of its action are seen in the distinction between *G. arboreum* var. *typicum* and var. *neglectum*. Geographical factors are responsible for the differences between the four forms and run parallel in var. *typicum* and var. *neglectum* from the same area. The differences are variable and primarily of agricultural interest. In the absence of information on origin and history no attempt should be made to assign material to a particular form. Forma *burmanica* and forma *bengalensis* have been influenced in the development of annual types primarily by the short season of northern India and China and Japan. Forma *bengalensis* has also given rise, in response to the special conditions of the hill tracts of Assam and Bengal, to var. *cernuum*. The origin of var. *neglectum* forma *bengalensis* and of var. *cernuum* is associated with an area of high variability in western Burma and Assam. In the tract bounded by the Bay of Bengal, and the Irrawaddy, Chindwin and Brahmaputra rivers are to be found types covering the whole range of variability of *neglectum bengalensis* and *cernuum* [Stock, 1927]. Forma *indica* has given rise to most of its annual types in quite recent times, in response to changed agricultural demands. While it is not possible to distinguish on botanical characters between many of the types of *G. arboreum* var. *neglectum* forma *bengalensis*, and *G. arboreum* var. *neglectum* forma *indica*, they are physiologically very different. When grown in northern India most strains of var. *neglectum* forma *indica* develop a bushy, monopodial habit similar to that of their var. *typicum* ancestors. Plate XII, fig. 2 shows such a monopodial plant of *G. arboreum* var. *neglectum* forma *indica* which was grown at Indore. This appears to be a response to length of day, and is strong evidence of an origin independent of that of forma *bengalensis*. Hutchinson and Govande [in press] have also shown recently that the difference in hair characters and spinning properties between forma *bengalensis* and forma *indica* are as great as between the three species *G. arboreum*, *G. herbaceum*, and *G. hirsutum*.

Introductions of seed of American species of cotton into India and Africa have been made on many different occasions and all three species have become established. Information concerning their status and survival value in India is scanty except for *G. hirsutum* (Upland), concerning the establishment of which an extensive literature exists. The greatest measure of success has been achieved in the Punjab with Punjab-American, and in Madras with 'Cambodia'. Upland cottons are to be found, however, scattered almost all over India as components of more or less importance in the local cotton crops. In Africa the cotton of Uganda, Tanganyika, Nyasaland, Rhodesia, the Union of South Africa, Northern Nigeria and the rain-fed tracts of the Sudan is produced from introduced forms of *G. hirsutum*. *G. religiosum* (Bourbon) has become established as a component of the perennial *Nadam* crop on some of the red soils of the Madras Presidency and Mysore. Scattered records exist of its occurrence in other parts of India. Forms of this species have also become established in Africa (Gambia and Southern Sudan) and Madagascar. Apart from the present-day attempt to establish Egyptian and Sea Island cottons in Sind, no success has been achieved in introducing *G. barbadense* as a crop in India. Occasional plants of perennial types, particularly the kidney-seeded type usually known as *G. braziliense*, have become established in gardens in various parts of India (Calcutta and Saharanpur Botanic Gardens, Central India, Central Provinces, Bombay and Madras Presidencies). It is of interest to note that in Central India and in Madras lint from kidney cottons is used for spinning the sacred thread of the Hindus, having replaced the indigenous cotton for this purpose. In Africa the cottons of Egypt and the irrigated tracts of the Sudan and of Southern Nigeria are forms of *G. barbadense*.

One other species of *Gossypium* requires brief mention. On the basis of two rather unsatisfactory specimens from near Gharo in the Karachi desert, Sind, Watt [1926] described a new species to which he gave the name *G. Bakeri*. There appear to be no other records of the species, though it is certainly distinct both from *G. Stocksii* and from the cultivated Indian species.

V. Summary

(1) The present state of knowledge concerning the classification of *Gossypium* species is summarised with particular reference to the literature on Asiatic cottons.

(2) A classification of the indigenous and naturalised cottons of India and Africa is developed on the basis of modern genetic and taxonomic knowledge.

(3) Cultivated Asiatic cottons are classified as follows :—

G. arboreum L. var. a. *typicum*
var. b. *neglectum*
var. c. *cernuum*

G. herbaceum L. var. a. *typicum*
var. b. *frutescens*
var. c. *africanum*

(4) *G. arboreum* var. *typicum* and *G. arboreum* var. *neglectum* are divided on geographical grounds, and on account of their recent evolutionary history, into four forms :—

I. forma *bengalensis*
II. forma *burmanica*
III. forma *indica*
IV. forma *soudanensis*

(5) Cultivated New World cottons are classified according to Harland's scheme into :—

G. hirsutum L.
G. religiosum L.
G. barbadense L.

(6) *G. Stocksii* Mast., the wild cotton of Sind and Arabia, and *G. anomalum* Wawra and Peyr., the wild cotton of Somaliland and the Sudan, are described and the parallelism between them and *G. herbaceum* L. and *G. arboreum* L. is discussed. *G. Bakeri* Watt, a little-known wild species from Sind, is mentioned. Reference is made to *Cienfugosia triphylla* Engler, which is transferred by Chevalier [1933], and Skovsted [1935] to *Gossypium* as *G. triphyllum*.

(7) The distribution of the Asiatic species and recent evolutionary trends in them are discussed.

(8) The present status of the New World species in India and Africa is summarised.

VI. Acknowledgments

The present classification is very largely the result of the authors' study of living cottons in the field and in experimental plots. In writing the paper and in discussing previous taxonomic work we have been greatly assisted by a study of the collection of cotton specimens in the herbarium of the Royal Botanical Gardens, Sibpur, Calcutta. We have pleasure in acknowledging our indebtedness to the Director of the Botanical Survey of India for his courtesy in permitting the loan of the collection to the Institute of Plant Industry.

Prof. Aug. Chevalier very kindly read the first draft of this paper and his discussion of his collections of African material in the light of our classification has thrown a flood of light on the status of *Gossypium* in Africa. We have taken full advantage of the information with which he has provided us, in revising our manuscript, and we gladly acknowledge our indebtedness to him.

We are also indebted to M. Perrier de la Bathie for identifying specimens of plants grown in Trinidad from Madagascar seed, for information concerning the type, and for a reference to Hochreutiner's description of it (as *G. herbaceum* var. *Perrieri*) in 'Candollea'.

Our information concerning the cottons of South India would have been inadequate for our purpose, had we not been able to draw freely upon the extensive knowledge of Mr. Ramanatha Ayyar, Cotton Specialist to the Madras Government. We also owe to him the plate of *G. religiosum*.

We are indebted to Dr. J. Burtt Davy, F.L.S., for much invaluable advice on nomenclature and on the arrangement and contents of the botanical descriptions. He has also very kindly criticised the manuscript and checked certain references which are not available in India.

REFERENCES

Chernyakovskaya, E. G. (1930). *Bull. Appl. Bot. Genet. and Pl. Breed.* **23** No. 5, 3.
Chevalier A. (1933). *Rev. Bot. Appl. Agric. Trop.* **13**, 190.
————(1935). *Ibid.* **15**, 369.
————(1936). *Ibid.* (In press).
Delile (1813). *Fl. Ægypt. Illustr.* **2**, 69.
Gammie, G. A. (1907). *Mem. Dept. Agric. Ind.* (*Bot. Ser.*) **2**, No. 2.
Harland, S. C. (1928). *Trop. Agric.* (*Trinidad*) **5**, 116.
————(1932). *Bibliogr. Genet.* **9**, 107.
————(1936). *Biol. Rev.* **2**, 1.
Hochreutiner (1924-6). *Candollea* **11**, 142.
Hooker, J. (1874). *Fl. Brit. Ind.* **1**, 346.
Hutchinson, J. B. (1932). *Jour. Genet.* **26**, 317.
———— (1934). *Ibid.* **28**, 437.
———— (1935). *Ibid.* **31**, 451.
———— (1936). *Ibid.* **32**, 399.
Hutchinson, J. B. and Govande, G. K. (In press).
Leake, H. M. and Ram Prasad (1914). *Mem. Dept. Agric. Ind.* (*Bot. Ser.*) **6**, 115.
Linnaeus, C. (1753). Sp. Plantarum.
———— (1763). Sp. Plantarum, Ed. 2.
———— (1766-8). *Syst. Nat.* **12**.
Patel, L. P. (1920). *Mem. Dept. Agric. Ind.* (*Bot. Ser*). **11**, No. 4.
Skovsted, A. (1935). *Jour. Genet.* **30**, 447.
Stock, T. D. (1927). *Mem. Dept. Agric. Ind.* (*Bot. Ser.*) **14**, 177.
Todaro, A. (1877). *Relaz. Cult. dei Cotoni.* (*Quoted by* Watt [1907] see below.)
Vavilov, N. I. (1926). *Bull. Appl. Bot. and Pl. Breed.* **16**, No. 2, 1.
Watt, G. (1907). " The Wild and Cultivated Cotton Plants of the World." London Longmans, Green & Co.
———— (1926). *Kew. Bull.* **5**, 193.
———— (1927). *Ibid.* **8**, 321.
Wawra and Peyritsch (1860). *Sert. Benguel.* **21**.
Zaitzev, G. S. (1928). *Bull. Appl. Bot. and Pl. Breed.* **18**, No. 1, 39.

STUDIES ON THE ROOT-ROT DISEASE OF COTTON IN THE PUNJAB

III. THE EFFECT OF SOME PHYSICAL AND CHEMICAL FACTORS ON SCLEROTIA FORMATION

BY

R. SAHAI VASUDEVA, B. Sc., Ph. D. (London), D. I. C.,

Assistant Cotton Mycologist, Department of Botany, Punjab Agricultural Research Institute, Lyallpur

(Received for publication on 1st September 1936)

(With three text-figures)

CONTENTS

PAGE
1. Introductory 259
2. Experimental method 260
3. Effect of various factors on the formation of sclerotia . . 260
    I. Effect of medium 260
    II. Effect of concentration of the medium 261
    III. Depth of the medium as affecting sclerotia formation . . . 262
    IV. Effect of pH value of the medium 262
    V. Effect of light 263
    VI. Effect of individual components of the medium 263
4. Growth of the *Rhizoctonia* spp. in synthetic media in relation to the effect of nitrogen from different compounds and the toxic effect of various chemicals on the fungi 264
    I. Growth in relation to the effect of nitrogen from different compounds 264
    II. Effect of formaldehyde and ammonia on mycelium in cotton roots . 266
    III. Effect of ammonium hydroxide on the sclerotia 266
    IV. Effect of mercuric chloride, copper sulphate and phenol on the growth 267
    V. Effect of hydrocyanic acid gas on the viability of the sclerotia . 268
5. Summary 269
References 270

1. Introductory

The experiments described in this paper were carried out with two organisms *Rhizoctonia bataticola** and *Rhizoctonia solani* Kuhn group (new strain) which have been found to cause root-rot disease of cotton [Vasudeva, 1935] in the Punjab. As the resting bodies of these fungi are very important from the point of view of 'carry over' of the disease, experiments were conducted to determine the conditions that favour the formation of these bodies. Also the toxic effect of various chemical compounds on the mycelium and sclerotia of the causal fungi has been investigated, in connection with the study of methods of control of the disease.

---

* The position regarding the specific name of the fungus has not yet been determined. It will be given in a later publication.

2. Experimental method

The behaviour of the two fungi, *R. bataticola* and *R. solani*, was studied in Petri dish cultures. Dishes of uniform size were used throughout the investigation and proper care was taken to pour almost uniform quantities of the medium throughout unless mentioned otherwise. The following media were used :—

(1) * Cotton root extract agar.—

Cotton roots	200 grams.
Distilled water to make up	1,000 c. c.

(2) Cotton root synthetic [Vasudeva, 1936].—

Glucose	20 grams
Peptone	2·9 ,,
K_3PO_4.	1·9 ,,
$Ca_3(PO_4)_2$	0·9 ,,
$MgSO_4$.	0·4 ,,
NaCl	0·6 ,,
$FeCl_3$	a trace.
Distilled water to make up	1,000 c. c.

(3) Richards' agar.

(4) Brown's agar.

Plates were, as a rule, inoculated at the centre after which they were wrapped in tissue paper to guard against contamination and incubated at 30°C. To ensure purity the work was started with single hyphal-tip cultures which were maintained on potato extract agar.

3. Effect of various factors on the formation of sclerotia

I. EFFECT OF MEDIUM

After six days' growth at 30°C., the following observations were recorded.

On cotton root extract and cotton root synthetic agar

R. bataticola.—Growth luxuriant and spreading, sufficient aerial mycelium Numerous tiny dark sclerotia formed scattered throughout the medium.

---

* Cotton roots were cut into small pieces covered with distilled water and autoclaved at 15 lbs. pressure for one hour and passed through muslin. The extract thu obtained was made up to 1,000 c. c. by adding distilled water.

R. solani.—Growth profuse, spreading, plenty of aerial mycelium. A large number of sclerotia present on the surface of the medium and also scattered throughout the aerial mycelium.

On Richards' agar

In the case of both R. bataticola *and* R. solani.—The growth same as that obtained on cotton root synthetic and cotton root extract but fewer sclerotia.

On Brown's agar

R. bataticola.—Growth good but not so widely spread, fair amount of aerial mycelium, cottony-white near the periphery, turning grey towards the centre. Sclerotia present though less numerous.

R. solani.—Growth profuse, plenty of white aerial mycelium and a few aggregations of hyphae forming into sclerotia.

The data are put in quantitative form for comparative study in Table I.

TABLE I

Effect of medium

Organism	Medium	Diameter of colony (Av. of 4 plates)	Sclerotia
		cm.	
R. bataticola .	Richards' agar . . .	11·5	++++
,, .	Cotton root extract agar . .	11·3	+++++++
,, .	Cotton root synthetic . .	11·2	+++++++
,, .	Brown's agar . . .	8·1	++
R. solani .	Richards' agar . . .	10·1	++
,, .	Cotton root extract agar . .	11·5	+++
,, .	Cotton root synthetic . .	11·2	+++
,, .	Brown's agar . . .	8·0	very few

The above table shows that the growth on Richards' agar and cotton root synthetic and extract agar is almost uniform but the sclerotia formation on the last two media is more luxuriant. On Brown's agar, however, the sclerotia formation is poor.

II. EFFECT OF CONCENTRATION OF THE MEDIUM

Further experiments were conducted in order to test the effect of concentration of the medium. For this Richards' agar of different strengths was used. If normal concentration of the medium be denoted by N, a range of concentrations from $8N$ to $N/16$ was tested.

The results of such an experiment conducted at 30°C. are given in Table II

TABLE II

Effect of concentration of the medium

Concentration of medium	*R. bataticola*		*R. solani*	
	Average diameter of colonies (4 days)	Sclerotia after 12 days growth	Average diameter of colonies (4 days)	Sclerotia after 12 days growth
8*N*	Slight growth	+++++++	No growth	–
4*N*	8·2 cm.	+++++	,,	–
2*N*	10·0 cm.	++++	2·1 cm.	–
N	10·7 cm.	++++	8·5 cm.	+++
N/2	9·0 cm.	+++	9·1 cm.	+
N/4	7·5 cm.	++	11·2 cm.	++
N/8	7·3 cm.	Contaminated	10·7 cm.	+
N/16	6·6 cm.	+	10·6 cm.	+

From the above table it appears that in the case of *R. bataticola* there is positive correlation between the concentration of the medium and the intensity of sclerotia formation.

The relation holds good for *R. solani* also but is not so marked.

III. DEPTH OF THE MEDIUM AS AFFECTING SCLEROTIA FORMATION

A simple method of demonstrating the effect of depth of medium on the sclerotia formation is to pour the plates in such a way that the medium grades from very shallow on one side to very deep on the other side.

This experiment was carried out on two media, *i.e.* cotton root synthetic and Richards' agar. The Petri dishes before pouring the medium were marked with Indian ink at the bottom so as to represent four distinct regions, two on either side of the inoculum which represented the centre of the plate.

In the case of *R. bataticola* the sclerotia formation was more pronounced on the deep medium, whereas the amount of sclerotia diminished with the depth of the medium and very few sclerotia were formed on the shallow medium.

The sclerotia of *R. solani* were formed at a distance of 3 cm. from the inoculum on the deep side of the medium only. On the shallow medium sclerotia were not formed.

IV. EFFECT OF *p*H VALUE OF THE MEDIUM

The effect of *p*H value on sclerotia formation was shown in some experiments in which cotton root synthetic medium was made up to different degrees of acidity and alkalinity by addition of malic acid or sodium bicarbonate. Nine modifications of the medium ranging in *p*H from 3 to 8·4 were made up. The sclerotia formation of *R. bataticola* and *R. solani* was studied side by side on these media. The results of such an experiment are set out in Table III.

TABLE III

Effect of pH *value on sclerotia formation*

*p*H (initial)	Sclerotia formation in *R. bataticola*	Sclerotia formation in *R. solani*
3·3	++++	++
3·8	++++	++
4·2	++++	Contaminated
6·8	++++	++++
7·6	++++	+++
7·8	++++	++
8·0	++	+
8·4	++	+
9·2	No growth	No growth

These results show that intense sclerotia formation occurs in the case of *R. bataticola* from *p*H 3·3 to 7·8. At *p*H 8 there is a considerable fall in sclerotia formation whereas the size of the sclerotia is much bigger than the normal.

R. solani shows its best sclerotia formation at *p*H 6·8 and on either side of this *p*H there is a gradual fall in the formation of sclerotia but this fall is more pronounced on the alkaline medium range.

V. EFFECT OF LIGHT

This was tested on three media, cotton root synthetic, Richards' agar and Brown's agar. Cultures of *R. bataticola* and *R. solani* were exposed to light and also kept in complete darkness. Very little difference in the formation of sclerotia was observed when the cultures were kept in light or in darkness.

VI. EFFECT OF INDIVIDUAL COMPONENTS OF THE MEDIUM

Experiments were carried out in which the concentration of individual components of a medium was varied or in which certain chemical compounds were replaced by compounds of similar nature. The medium used in such experiments was cotton root synthetic.

The three constituents, sodium chloride, magnesium sulphate and ferric chloride are not of great importance. When these are omitted the type of growth and sclerotia formation are almost unchanged. The other constituents, *i.e.* carbohydrate, nitrogen and phosphorus, are important and materially affect the growth and sclerotia formation as given below.

Sugar.—Variation in the amount of sugar produces marked effects. When this is omitted, the growth is very poor. The mycelium is thin and spreading and there is hardly any sclerotia formation in the case of *R. bataticola* and *R. solani.* As the concentration of sugar is increased, even beyond the normal amount, the growth improves and the sclerotia formation becomes more intense. Chona [1932] obtained similar results while studying the conditions for the formation of perithecia of the *Aspergillus glaucus* series.

Nitrogen source.—In the absence of peptone the growth is very poor and scanty and there is no sclerotia formation in the case of both the fungi.

When peptone is replaced by ammonium sulphate or ammonium chloride, calculated to yield the same amount of nitrogen, growth and sclerotia formation is as good as in normal cotton root synthetic. But the size of the sclerotia formed in the presence of ammonium salts is much larger. When peptone is replaced by potassium nitrate the sclerotia formation is considerably reduced.

Phosphatic constituent.—When this is omitted, the vigour of growth is reduced and sclerotia are altogether absent. When potassium phosphate is replaced by potassium-dihydrogen phosphate the sclerotia formation is as good as in the normal medium. But if potassium phosphate is replaced by magnesium phosphate the sclerotia formation is comparatively poor. This is probably due to the absence of potassium. When the combination, peptone+potassium phosphate+calcium phosphate, is replaced by ammonium chloride+potassium-dihydrogen phosphate, no difference in the amount of growth or sclerotia formation is noticed.

Tyner and Sanford [1935] also observed that sclerotia of *R. solani* Kuhn, were not produced when either phosphorus or nitrogen were absent from the medium.

4. Growth of the *Rhizoctonia* spp. in synthetic media in relation to the effect of nitrogen from different compounds and the toxic effect of various chemicals on the fungi.

I. GROWTH IN RELATION TO THE EFFECT OF NITROGEN FROM DIFFERENT COMPOUNDS

R. bataticola and *R. solani* were grown in cotton root synthetic solutions to which the requisite amounts of nitrogen compounds had been added in order to yield 9·20 grams of nitrogen per litre. These cultures were prepared in Erlenmeyer flasks of 100 c.c. capacity containing 30 c.c. of medium. As far as possible inocula of uniform size were introduced into each flask after sterilization. Hydrogen-ion concentrations of un-inoculated control solutions were determined soon after sterilization, and the dry weights of fungal mycelium and *p*H of the stales thus obtained were taken at suitable intervals. The fungal mats were collected on filter papers by means of a Buchner filter, dried to constant weight in a hot air oven, cooled over calcium chloride in a desiccator and weighed. The *p*H values were determined by the colorimetric method described by Clark [1922]. The results of such experiments are given in Tables IV and V.

TABLE IV

Weight of mycelium of R. bataticola *and* p*H of fungal stales after 12, 20 and 27 days' growth at 25° C. in cotton root synthetic containing different nitrogen carriers yielding 9·20 grams of nitrogen per litre*

Source of nitrogen added	*Average dry weight (in mg.) of fungal mats after			*p*H values of controls	*p*H values of stales		
	12 days	20 days	27 days		12 days	20 day	27 days
Peptone . .	336	281	200	6·4	8·0	8·0	8·0
KNO_3 . . .	222	380	263	6·8	7·0	8·0	8·0
$Ca(NO_3)_2$ . .	399	345	422	5·4	5·0	6·2	6·6
$NH_4 NO_3$ . .	54	205	113	6·6	5·0	6·4	6·6
$(NH_4)_2 SO_4$ . .	238	231	184	6 4	5·0	3·2	3·7

TABLE V

Weight of mycelium of R. solani *and* p*H of fungal stales after 10, 17, and 27 days' growth at 25° C. in cotton root synthetic containing different nitrogen carriers yielding 9·20 grams of nitrogen per litre*

Source of nitrogen added	*Average dry weight (in mg.) of fungal mats after			*p*H values of controls	*p*H values of stales		
	10 days	17 days	27 days		10 days	17 days	27 days
Peptone . .	417	246	284	6·4	8·0	8·2	8·0
KNO_3 . . .	52	277	223	6·6	7·0	7·2	7·8–8·0
$Ca (NO_3)_2$ . .	82	408	389	5·4	6·2	6·6	7·2
NH_4NO_3 . .	246	195	145	6·4	4·8–5·0	7·0	7·0
$(NH_4)_2 SO_4$ . .	160	158	122	6·4	2·8	2·8	2·8

In tests of various sources of nitrogen no appreciable advantage for any of the nitrogen carriers tested was observed, but the cultures containing the ammonium salts as nitrogen carriers showed a toxic effect on the growth of these fungi. Calcium nitrate and peptone when supplied as nitrogen sources produced the best fungal growth. The filtrates in the case of ammonium sulphate became very acid whereas there was increase in alkalinity following decomposition of potassium nitrate.

---

* Average of three cultures.

When the quantities of these nitrogen sources were increased so as to yield 18·4 grams of nitrogen per litre, the growth in all cases was reduced a great deal excepting where peptone was used.

II. EFFECT OF FORMALDEHYDE AND AMMONIA ON MYCELIUM IN COTTON ROOTS

Cotton roots were inoculated with *R. bataticola* and *R. solani* and after the growth of the fungi had been well established, the inoculated roots were exposed to formaldehyde vapour for thirty seconds. The roots thus treated were then transferred to cotton root extract agar. It was observed that the mycelium in the treated roots was killed whereas the mycelium in the controls remained viable. When diseased roots were treated with ammonia similar results were obtained.

III. EFFECT OF AMMONIUM HYDROXIDE ON THE SCLEROTIA

In an experiment to test the effect of ammonium hydroxide on the sclerotia of *R. bataticola* and *R. solani* the resting bodies of the fungi were exposed in concentrations of ammonium hydroxide ranging from 0·5 to 3·0 per cent for various intervals, *i.e.*, one to twenty minutes. Controls were kept side by side and were exposed to sterile water for the same length of time.

The sclerotia had been cultivated on cotton root synthetic agar. After exposing the sclerotia to ammonium hydroxide they were given a thorough washing in sterile water, transferred to cotton root extract agar and incubated at 30°C. in order to test their viability. The germination results of the sclerotia so treated are given in Table VI.

TABLE VI

Effect of ammonium hydroxide on the sclerotia

Fungus	Time of exposure in minutes	Viability of sclerotia exposed to ammonium hydroxide concentration					
		0·5 per cent	1·0 per cent	2·0 per cent	3·0 per cent	4·0 per cent	5·0 per cent
R. Solani	1	100	100	26·6	6·6	..	..
	5	100	60	26·6	0	..	..
	10	100	80	15·3	0	..	..
	20	100	26·6	0	0	..	..
R. bataticola	1	100	100	100	100	100	100
	5	100	100	100	100	86·6	46·6
	10	100	100	100	100	0	0
	20	100	100	100	93·3	0	0

The results obtained show that the resting bodies of *R. solani* lose their viability when exposed to three per cent ammonium hydroxide solution for five minutes whereas ten minutes exposure to four per cent ammonium hydroxide is required to kill the sclerotia of *R. bataticola*. Controls gave cent per cent germination.

IV. EFFECT OF MERCURIC CHLORIDE, COPPER SULPHATE AND PHENOL ON THE GROWTH

The toxic concentrations of these chemical compounds were determined by growing *R. bataticola* and *R. solani* on Brown's agar to which varying amounts of mercuric chloride, copper sulphate or phenol had been added.

The comparative growth rates of both the fungi on media containing various dilutions of mercuric chloride, copper sulphate and phenol are shown in Figs. 1, 2 and 3, respectively.

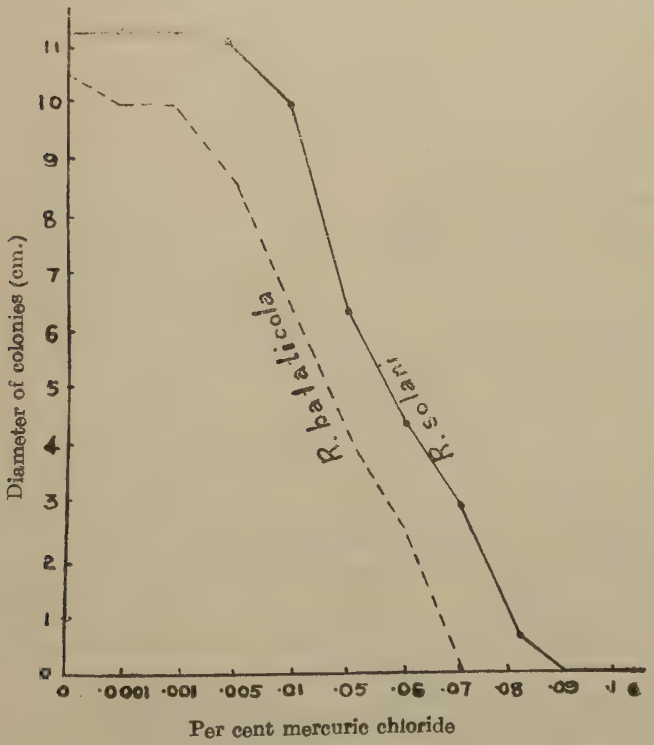


Fig. 1.—Growth rates of *Rhizoctonia bataticola* and *R. solani* on Brown's agar containing various dilutions of mercuric chloride.

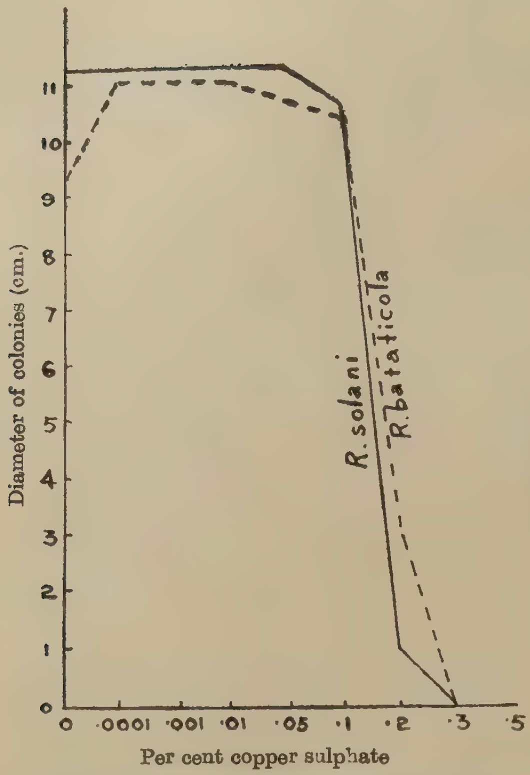


Fig. 2.—Growth rates of *Rhizoctonia bataticola* and *R. solani* on Brown's agar containing various dilutions of copper sulphate.

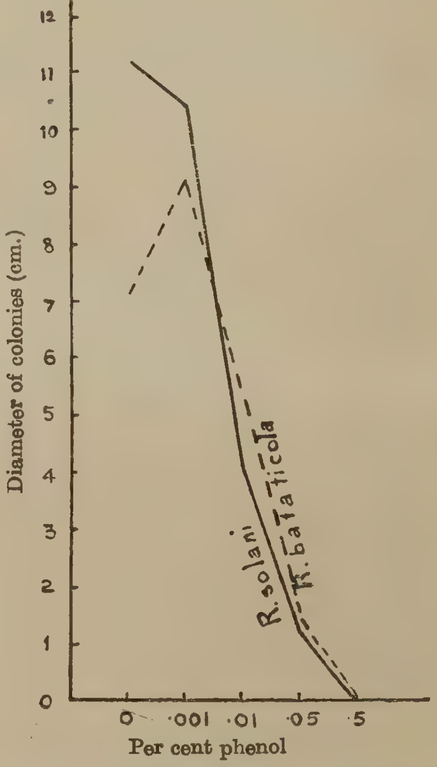


Fig. 3.—Growth rates of *Rhizoctonia bataticola* and *R. solani* on Brown's agar containing various dilutions of phenol.

It is clear from the figures that 0·09 per cent mercuric chloride, 0·30 per cent copper sulphate and 0·50 per cent phenol solutions are quite effective in checking the growth of both the fungi.

V. Effect of hydrocyanic acid gas on the viability of the sclerotia

In order to test the effect of hydrocyanic acid gas on the sclerotia an air-tight chamber containing potassium cyanide fixed at the bottom by means of Plaster of Paris was used. Sclerotia of the causal organisms were obtained from diseased cotton roots, placed in a watch glass and exposed in dry as well as in moist condition to the hydrocyanic acid gas in the chamber for intervals ranging from fifteen minutes to one week.

It was observed that the sclerotia exposed in moist condition for forty-five minutes to hydrocyanic acid gas failed to grow, whereas those exposed in dry condition were found to be viable even after a week. Controls kept under normal conditions gave cent per cent germination.

In another experiment tests were carried out to determine the penetrating power of hydrocyanic acid gas in the soil. The toxic effect on the sclerotia of *R. bataticola* and *R. solani* at a certain depth in the soil was taken as an index of penetration of the hydrocyanic acid gas to that depth.

Glass tubes, one inch in diameter and forty inches in length, were used for studying the toxic effect of the gas on the sclerotia at various depths in the soil. These glass tubes were washed with alcohol, dried and flamed. The tubes were then filled with moist sterilized soil in six-inch layers and bits of cotton roots with sclerotia were also placed in these layers. The lower end of the tube was then stoppered with a sterilized cork and calcium cyanide added at the other end of the tube and thoroughly packed up with sterilized soil.

This was carried out in duplicates. In controls calcium cyanide was not added. Viability tests of pieces of cotton roots with sclerotia were carried out at regular intervals. The results of such an experiment are given in Table VII.

TABLE VII

Effect of hydrocyanic acid gas on the sclerotia buried in moist soil at various depths

Time of exposure in days	Viability percentage of sclerotia at various depths					
	6 in.	12 in.	18 in.	24 in.	30 in.	36 in.
2 . . .	0	13	100	100	100	100
3 . . .	0	8	100	100	100	100
4 . . .	0	8	100	100	100	100
6 . . .	0	0	0	100	100	100

The data obtained show that hydrocyanic acid gas penetrates up to a depth of eighteen inches after six days and kills the resting bodies of the causal fungi. Controls in all cases gave cent per cent germination. The experiment was repeated a number of times and similar results were obtained.

The author is greatly indebted to Professor J. C. Luthra, for valuable help and criticism throughout the course of this investigation. He is also grateful to Sir B. C. Burt, Vice-Chairman, Imperial Council of Agricultural Research, for his suggestions in connection with the experiments with hydrocyanic acid gas. Thanks are also due to Ch. M. Ashraf for the assistance rendered.

5. Summary

(1). The intensity of sclerotia formation of *R. bataticola* and *R. solani* depends on the concentration, depth and the pH value of the medium.

(2) The importance of sugar, nitrogen source and phosphatic constituent on the formation of sclerotia has been explained.

(3) Of the various salts used for sources of nitrogen the cultures containing the ammonium salts have a toxic effect on the growth of the fungi studied.

(4) Mercuric chloride, 0·09 per cent, copper sulphate, 0·30 per cent and phenol, 0·50 per cent solutions are quite effective in checking the growth of the fungi.

(5) Sclerotia of the fungi when exposed in moist condition for forty-five minutes to hydrocyanic acid gas lose viability, but under dry condition the sclerotia are viable even after one week's exposure.

(6) Hydrocyanic acid gas penetrates in the soil up to a depth of eighteen inches after six days and kills the resting bodies of both the fungi.

REFERENCES

Chona, B. L. (1932). *Trans. Brit. Myc. Soc.* **17**, 221-228.
Clark, W. M. (1922). 'The Determination of Hydrogen Ions'.
Tyner, L. E. and Sanford, G. B. (1935). *Sci. Agric.* **16**, 197-207.
Vasudeva, R. Sahai (1935). *Ind. Jour. Agric. Sci.* **5**, 496-512.
——————— (1936). *Ind. Jour. Agric. Sci.* **6**, 904-916.

A COMPARATIVE STUDY OF SPECIES OF *SEPTORIA* OCCURRING ON WHEAT

BY

JAI CHAND LUTHRA, M.Sc., D.I.C. (Lond.)
Professor of Botany
ABDUS SATTAR, B.Sc. (Ag.), Assoc. I.A.R.I., Ph.D (Lond.), D.I.C. (Lond.)
Assistant Mycologist

AND

M. ABDUL GHANI, M.Sc. (Ag.)
Punjab Agricultural College and Research Institute, Lyallpur

(Received for publication on 17th November 1936)

(With Plates XV–XVII and three text-figures)

CONTENTS

	Page
I. Introductory	271
II. Historical	272
III. Experimental	272
A. Material and methods	272
B. Description of disease symptoms and general morphological characters of the fungi on the host	273
C. Study of the various fungi under conditions of artificial culture	275
(*a*) Macroscopic growth features	275
(*b*) Microscopic growth features	279
(*c*) Germination of pycnospores	283
D. Inoculation experiments	285
IV. Discussion of results	287
V. Summary	288
References	289

I. Introductory

The *Septoria* disease of wheat which is reported to be widespread in Europe, Asia, Australia and America, first attracted the attention of the authors when the disease appeared in a serious form in some fields at the Agricultural Farm, Lyallpur. Later on, the disease was observed at Rawalpindi, Gurdaspur, Amritsar, Jullundur and Hoshiarpur. The uncertainty of the identity of the causal fungus and the importance of the disease led to the investigation reported in this paper.

II. Historical

The fungus causing this leaf-spot disease of wheat was first described by Desmazieres [1842] as *Septoria tritici*. In 1843 he described *Septoria graminum* on leaves of grasses stating that it was very similar to *Septoria tritici*, the only distinguishing feature being that in *Septoria graminum* the pycnidia (called perithecia by him) were smaller and closer set and the pycnospores narrower and rather enlarged at one end than in *Septoria tritici*. According to Erikson [1891] *Septoria tritici* was reduced to varietal rank under *Septoria graminum* making it *Septoria graminum* var. *Septoria tritici*. This usage was subsequently followed and many have applied the species name alone (*Septoria graminum*) to the fungus on wheat. Weber [1922] concluded that the form on wheat was different from *Septoria graminum* and that the appropriate name for this should be *Septoria tritici*.

Still another *Septoria* on the nodes and internodes of wheat was first described by Berkeley [1845] as *Septoria nodorum*. Passerini [1877] described a fungus on glumes of wheat in Italy as *Septoria glumarum*. Kuhn [1878] described an organism on the glumes of wheat in Germany as *Phoma hennebergii*, which later Berlese and Voglino [1886] found to be *Macrophoma hennebergii* and still later Voglino [1904] thought that it should be included with *Septoria glumarum*. Grove [1916] after examining the material thought that *Septoria nodorum* and *Septoria glumarum* were identical and called the fungus *Septoria nodorum*. Rosen [1921] confirmed Grove's [1916] description and then later Weber [1922] concluded that the fungus found on glumes of wheat in U. S. A. was *Septoria nodorum*.

Voglino [1904] in certain cultures of *Septoria nodorum* got the perithecial stage which he identified as *Sphœrella exitalis* Morini. Davies [1919] found perithecia associated with pycnidia of *Septoria nodorum* and placed it in the genus *Sphœrulina*. Weber [1922] found some perithecia closely associated with pycnidia of *Septoria nodorum* and identified them as belonging to *Leptosphœria* but, as the ascospores were not viable, he could not establish the relationship between the two.

In India *Septoria tritici* was collected on wheat at Lyallpur by *S. N. Mitra in 1905, the specimen being later identified and recorded by Sydow and Butler [1916]. Butler [1918] suspected that *Septoria tritici* Desm., *Septoria graminum* Desm., and *Leptosphœria tritici* (Grove) Pass., might probably be identical.

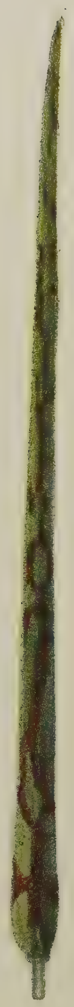
III. Experimental

A. MATERIAL AND METHODS

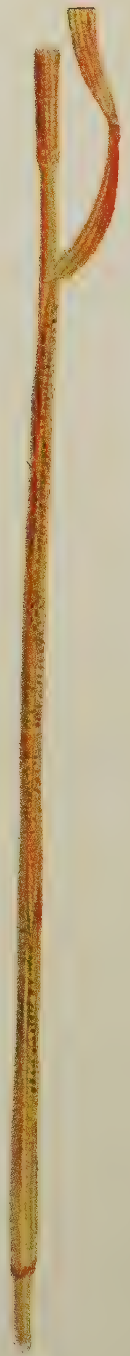
Diseased leaves, stem and ears of wheat collected at Lyallpur (Punjab) in the years 1933 to 1935, exsiccati of *Septoria tritici* Desm. received from Dr. E. J. Butler (England) and Messrs. L. R. Tehon (Illinois), W. W. Mackie (California)

---

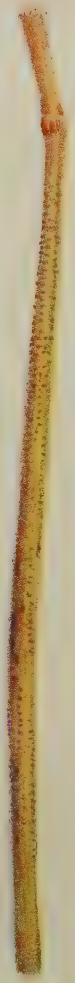
* Ascertained from the record of the Mycological Herbarium at the Imperial Agricultural Research Institute, New Delhi.



a



b



c

FIG. *a*. Wheat leaf affected with the local species of *Septoria*.
FIG. *b*. Wheat leaf sheath affected with the local species of *Septoria*.
FIG. *c*. Wheat stem affected with the local species of *Septoria*.

and H. C. Stenning (New South Wales) and exsiccati of *Septoria nodorum* Berk. received from Messrs. H. C. Stenning (New South Wales) and G. J. L. Burton (Kenya) have been studied.

The fungi included in the study are as listed below. For convenience of description the various fungi will be referred to under the names given within brackets.

1. *A Fungus isolated from lesions on wheat leaves at Lyallpur in 1933.*—This was found to resemble the fungus which was isolated from leaf-sheath, stem, and awns of wheat in 1935 at Lyallpur (*S. local*).

2. *Septoria tritici* Desm.—A culture obtained from the Centraalbureau Voor Schimmelcultures, Baarn, Holland in 1933 (*S. tritici*).

3. A fungus (*Septoria nodorum* Berk.) isolated from the lesions on wheat glumes (*Triticum vulgare*) obtained from Nojoro, Kenya, Africa in 1933 through Mr. G. J. L. Burton. This has been found to be identical with the culture of *Septoria nodorum* Berk. obtained in January 1935, from Centraalbureau Voor Schimmelcultures, Baarn, Holland (*S. nodorum*).

At the beginning of the work, the purity of all these cultures was ensured by the method of monosporal culturing.

The fungi were cultivated both in tubes and on plates on oat-meal agar, Richard's agar, Brown's agar, Brown's starch agar, potato dextrose agar, Czapek agar, onion agar and wheat-leaf-decoction agar.

B. DESCRIPTION OF DISEASE SYMPTOMS AND GENERAL MORPHOLOGICAL CHARACTERS OF THE PARASITES ON THE HOST

(*a*) *Symptoms*

S. local.—When the disease first appears, a slight mottling, barely noticeable, of the previously uniform green colour takes place ; then it becomes conspicuous as more or less circular or oval irregular spots, scattered over the leaf blades. The spots do not stand out prominently and it is only on account of the presence of thickly dotted black pycnidia that these are noticeable. Heavily infected leaves studded with pycnidia die prematurely. As the new leaves develop they also become infected. The leaf-sheaths are also commonly attacked and show the same symptoms.

On the stems and awns of wheat, the disease was noticed for the first time in 1935 at Lyallpur. On these parts no definite spots are formed and only pycnidia develop.

In the case of heavy infections young plants may totally dry up, otherwise only the infected leaves die. The disease helps in aggravating the attack of rusts in causing shrivelling of grains. (Affected leaf, leaf-sheath and stem are shown in Plate XV).

S. tritici.—This is similar to *Septoria local* with the difference that it has not been reported on the stems and awns of wheat. Artificial inoculation experiments at Lyallpur have, however, shown that it can attack the stem.

S. nodorum.—This fungus is characterised by producing small, irregular, chocolate-brown spots which are studded with black pycnidia and are usually found on the upper half of the glumes. In the case of a severe attack the grains shrivel.

(*b*) *Size of pycnidia*

The data under this heading, as based upon the measurement of fifty pycnidia of each fungus are given in Table I.

TABLE I

Size of pycnidia and pycnospores in microns of the various fungi on the host plant

Fungus	Nature of material	Pycnidia		Pycnospores	
		Limits of variation	Average	Limits of variation	Average
S. local	Leaves of one-month-old plants.	35·3-137·2 × 35·3-105·9	100·7 × 71·7	19·6-47·1 × 1·56-1·96	36 × 1·68
,,	Leaves of 1½-months old plants.	89-215·6 × 78·4-149	141·4 × 96·7	33·3-70·6 × 1·96-3·92	49·8 × 2·78
,,	Leaves of 2½-months old plants.	78·4-196 × 58·8-137	147·8 × 95	35·3-80·4 × 1·96-3·92	50 × 2·92
,,	Leaves of 3½-months old plants.	86·3-176 × 59-137	152·9 × 104	35·3-74·5 × 1·96-3·92	55·3 × 2·9
,,	Leaves of 5-months old plants.	102-215 × 68-160	157 × 109·2	28·0-74·0 × 1·2-3·8	55·2 × 2·6
	Stem . . .	72-148 × 92-208	152 × 104	32·0-74·0 × 1·6-3·6	58 × 2·5
	Awns . . .	72-140 × 90-200	148 × 104	32·0-72·0 × 1·6-3·6	57 × 2·4
S. tritici	Leaves of 5-months old plants.	152-220 × 32-132	140·6 × 91·8	36·0-80·0 × 1·2-3·8	61·9 × 2·5
S. nodorum	Glumes . . .	48-200 × 48-148	109·8 × 92·0	14·0-32·0 × 2·8-4·4	21·3 × 3·6

It is clear from the figures given in Table I that pycnidia of *S. local* and *S. tritici* have approximately the same size and are both bigger than those of *S. nodorum*. The other fact brought out from the Table is that the pycnidia of *S. local* are smaller in size on young plants.

(c) *Size of pycnospores*

The size of pycnospores of the various fungi based on 100 measurements is also given in Table I.

It is clear that the pycnospores of *S. local* and *S. tritici* are approximately equal in size and that their length is about double and width about three-fourth of these dimensions in *S. nodorum*. It is also evident that the pycnospores of *S. local* are smaller on young plants.

The ratio length/width of spores of *S. nodorum* is only about 6 but that of *S. local* and *S. tritici* is about 22 and 24 respectively.

(d) *Septation of pycnospores*

The degree of septation of spores of the three fungi based upon a count of 1000 spores in each case is given in Table II.

TABLE II

The degree of septation of pycnospores of the various fungi on the host plant (percentages of spores of different types of septation)

Fungus	0	1	2	3	4	5
S. local	0	2	10	63	25	0
S. tritici	0	0	5	35	45	15
S. nodorum . . .	0	20	25	55	0	0

The pycnospores of *S. local* are from 1-4 septate and most of the spores are 3-septate. The pycnospores of *S. tritici* are 2-5 septate and most of them are 3-4 septate and those of *S. nodorum* are 1-3 septate.

C. STUDY OF THE VARIOUS FUNGI UNDER CONDITIONS OF ARTIFICIAL CULTURE

The different fungi listed in section III A were subjected to a lengthy series of cultural experiments with a view to a better understanding of their physiology and of their variability under different environmental conditions.

(a) *Macroscopic growth features*

(i) *Linear growth-rate under different environmental conditions*

The effect of depth of the medium and light on the linear growth of these fungi was tested and found to be unimportant.

Growth on different culture media.—The fungi were grown on eight media at 20°C. The linear growth rate was measured after twenty-five days of inoculation and is shown in Fig. 1.

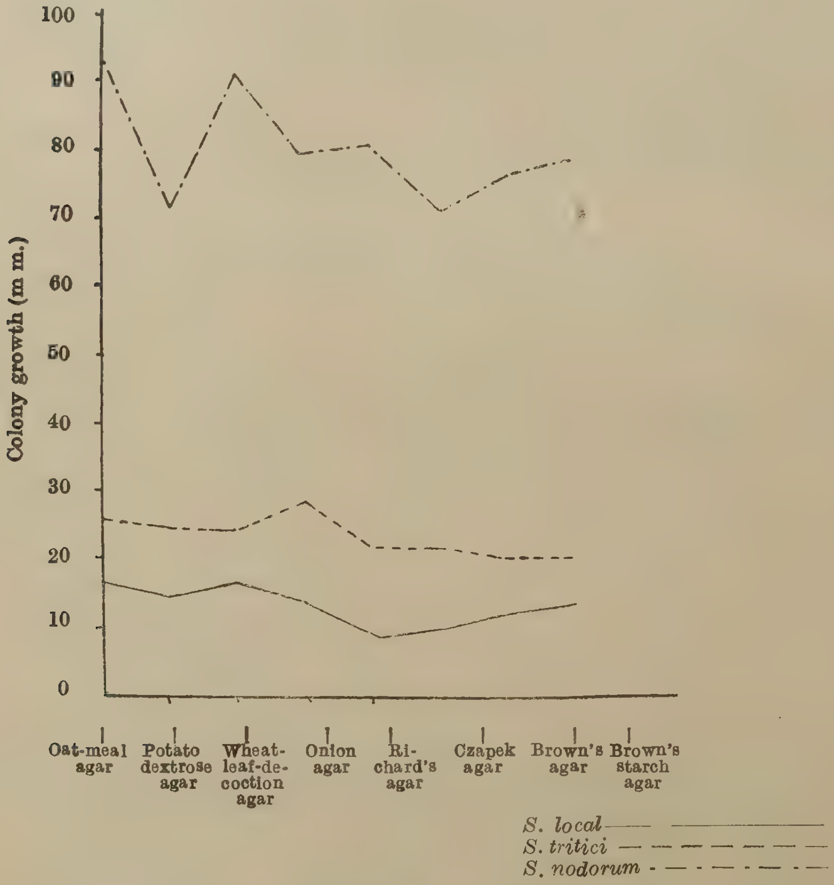


Fig. 1. Colony growth on different culture media at 20°C. after 25 days' growth.

The figure shows that the amount of linear growth of *S. local* is greater on natural than on synthetic media. The growth of *S. tritici* though better on natural media is also fair on the synthetic media employed. *S. nodorum* grows fairly well on all the media. It is also evident that *S. nodorum* grows faster than the other two fungi on all the media employed.

Temperature.—For this experiment wheat-leaf-decoction, Richards' and potato dextrose agars were employed, and the fungi were grown at 5 to 40°C. The observations were made after twenty days. The results on all the three media were almost the same. The linear growth on Richard's agar at different temperatures is shown in Fig. 2.

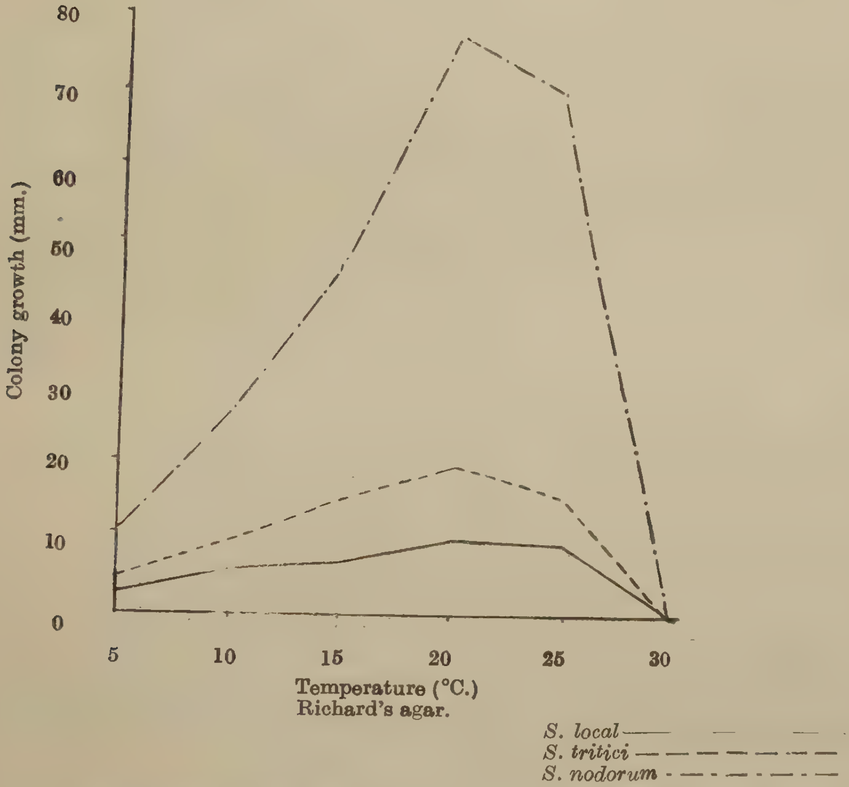


Fig. 2. **Effect of temperature upon colony diameter, after 20 days' growth on Richard's agar.**

It is clear from the results that the maximum temperature for growth of all the fungi is between 25 and 30°C., the minimum below 5°C. and the optimum is about 20°C.

It is also again evident that *S. nodorum* grows faster than the other two fungi within the limits of the experiment.

Hydrogen-ion concentration.—Throughout the experiment Richard's agar was employed as a medium. The various acid-alkali reactions were obtained by the addition of normal hydrochloric acid or sodium hydroxide, and the resulting *p*H's were determined colorimetrically by Clark's [1923] method. The fungi were grown at 20°C. and the observations were recorded after twenty days. The results are shown in Fig. 3.

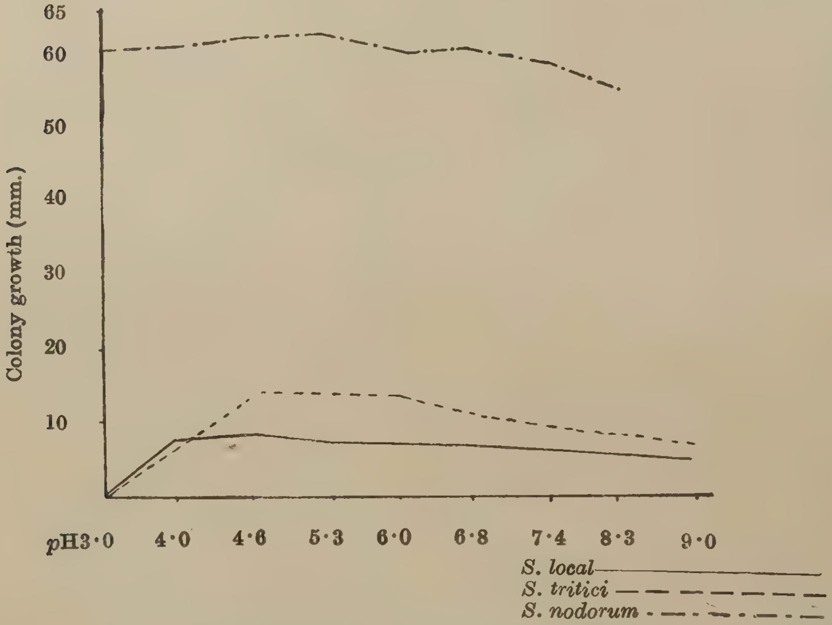


Fig. 3. Effect of *p*H on colony growth (20 days' growth at 20°C. on Richard's agar).

The results show that although the greatest linear growth of *S. local* is at *p*H 4·6, it grows fairly well between the ranges *p*H 4·0 to 8·9. The growth is checked on the acid side at *p*H 3·0.

The optimum *p*H for growth of *S. tritici* is at *p*H 4·6 to 6·0 ; on the alkaline side the growth is retarded at *p*H 9·0 and on the acid side the growth stops at *p*H 3·0.

S. nodorum grows well at a very wide range of *p*H, its growth being almost equal between *p*H 3·0 and 8·3. The growth is reduced at *p*H 9·0.

Here again it is clear that *S. nodorum* grows faster than the other two fungi.

(*ii*) *Aerial mycelium*

The degree of development of aerial mycelium varies with each fungus according to the environmental conditions. The amount of aerial mycelium produced by the various fungi on different media is represented in Table III.

TABLE III

Amount of aerial mycelium of the fungi on different culture media

Fungus	Oat-meal agar	Potato dextrose agar	Wheat-leaf-decoction agar	Onion agar	Richard's agar	Czapek agar	Brown's agar	Brown's starch agar
S. local	— to +	—	+	—	—	—	—	—
S. tritici	++++	++to +++	++++	—	—	—	—	—
S. nodorum	++++	++++	++++	++++	++++	++++	++	++

— means almost *nil* ; + means presence of aerial mycelium.

(*iii*) *Colony colour*

The colour of these fungi is the resultant of the colour of their respective pycnidia, spore exudate, and aerial mycelium and is affected by the variation in their respective amounts. Table IV gives the colours of the fungi on various media.

TABLE IV

**Colony colour of the fungi on eight culture media*

Fungus	Oat-meal agar	Potato dextrose agar	Richard's agar	Wheat-leaf-decoction agar	Onion agar	Czapek agar	Brown's agar	Brown's starch agar
S. local	Black	Pink to black	Blackish brown	Black to tan black	Pink	Blackish brown	Blackish brown	Blackish brown
S. tritici	White	White	White to black mouse grey	White	White to pink	White to hair brown	Tillent buff	Tillent buff
S. nodorum	White	White to violet grey	White	White	White	White	White	White

* as in Ridgeway.

(*b*) *Microscopic growth features*

(*i*) *Sporulation.*—*S. tritici* and *S. nodorum* form only one kind of spores, *i.e.*, pycnospores which are in pycnidia. *S. local* forms conidia also in addition to pycnospores.

The amount of sporulation of these fungi varies on different media and is greatly influenced by different environmental conditions. Thus *S. local* forms both pycnospores and conidia in abundance on oat-meal and wheat-leaf-decoction agars ; but on the other media tried, it has formed only conidia. It is interesting to note that the pycnidia of this fungus formed on oat-meal and wheat-leaf-decoction agars are also imperfect. On these two media pycnidia are formed on the surface of the medium after about ten to twelve days' growth and conidia are noticeable on the eighth or ninth day.

S. tritici forms pycnidia on potato dextrose, wheat-leaf-decoction and onion agars. On the other media tried pycnidia have not been observed even after twenty-five days' growth. On oat-meal agar it generally does not form pycnidia but on one occasion pycnidia were observed on this medium as well. In the case of potato dextrose agar pycnidia are visible, but in wheat-leaf-decoction, onion and oat-meal agars these are covered under the aerial mycelium. This fungus has not produced conidia. The pycnidia are formed after fifteen days on potato dextrose agar, after twenty days on wheat-leaf-decoction and onion agars. The pycnidia are more abundant on potato dextrose agar than on other media. The colour of the spore exudate is pink.

S. nodorum was not observed to form any pycnidia or conidia even after four months' growth at 20°C. in culture tubes or dishes, during the first eighteen months of the experiment. Then incidently it was discovered that in dishes of oat-meal and potato dextrose agars which had been kept in a Kelvinator at 8-10°C. the pycnidia formed after three months, whereas the dishes of the same media which were kept at room temperature (20°C.) did not show any signs of pycnidia formation even after six months' growth. The pycnidia were submerged in the substratum. They were pink on oat-meal agar and black on potato dextrose agar.

The amount of sporulation of the three fungi on different culture media is shown in Table V.

TABLE V

Amount of sporulation of the fungi on different culture media

Fungus	Oat-meal agar	Potato dextrose agar	Wheat-leaf-decoction agar	Onion agar	Richard's agar	Czapek's agar	Brown's agar	Brown's starch agar
S. local . . .	+++ to ++++	+++*	+++*and ++++	+++*	++* to +++*	++*	++*	++*
S. tritici . .	++†	++++	++	++	—	—	—	—
S. nodorum . .	++	++	—	—	—	—	—	—

— means almost *nil* ; + means presence of sporulation ; * means conidia ; † means pycnidia formed once only.

The effect of environmental conditions on sporulation has also been studied and the temperature has been found to have some influence. The amount of sporulation of *S. local* is less at 5°C. than at other temperatures, *S. tritici* does not sporulate at 5°C. and *S. nodorum* has been observed to form pycnidia at 10°C. only after three months' growth.

(*ii*) *Size of pycnidia*.—The pycnidia of *S. local*, as has already been mentioned, are imperfect and very loose. These, therefore, could not be measured. The size of the pycnidia of the other two fungi is given in Table VI. It is clear that the pycnidia of *S. nodorum* are smaller than those of *S. tritici*.

TABLE VI

Size of pycnidia, in microns, of the fungi on different culture media (based on 50 measurements in each case)

Fungus	Medium	Limits of variation	Average
S. tritici	Potato dextrose agar	137·2-294×98-225·2	227·4×152·9
S. nodorum	Do.	117·6-225·2×98-196	176·4×137·2
S. tritici	Oat-meal agar	140-294×98-230	225×165
S. nodorum	Do.	137·2-254·8×84-215·6	196×146·8
S. tritici	Wheat-leaf-decoction agar	137·2-294·0×98-225·2	227·4×152·9
S. tritici	Onion agar	129·4-278·4×98-227·4	156·8×215·6

(*iii*) *Size of pycnospores.*—The size of pycnospores of the various fungi on different culture media is given in Table VII.

It is clear that the pycnospores of *S. tritici* are somewhat longer than those of *S. local* and the pycnospores of *S. nodorum* are less than half the length of those of *S. tritici.*

It will be observed that the pycnospores of *S. local* are slightly longer on oat-meal than on wheat-leaf-decoction agar and those of *S. tritici* are longer on potato dextrose, oat-meal, and wheat-leaf-decoction agars than on onion agar.

TABLE VII

Size of pycnospores, in microns, of the fungi on different culture media

Fungus	Medium	Limits of variation	Average
S. local	Oat-meal agar	39·2-76·5×2·14-3·9	55·3×3·8
S. tritici	,, ,, ,,	28·4-104·9×1·57-3·9	66·6×2·0
S. nodorum	,, ,, ,,	12·51-23·5×3·14-3·9	22·3×3·8
S. local	Wheat-leaf-decoction agar	31·4-64·7×2·14-3·9	48·3×3·2
S. tritici	Do.	35·3-109·8×2·14-3·9	66·4×3·1
S. tritici	Potato dextrose agar	27·4-101·9×1·57-3·9	66×2·1
S. nodorum	Do.	13·7-23·9×3·53-4·3	19·9×3·9
S. tritici	Onion agar	35·3-82·3×1·57-2·3	46·6×1·7

TABLE VIII

Septation of pycnospores on different media

Fungus	Medium	0-Sept.	1	2	3	4	5	6	7
S. local . . .	Oat-meal agar . .	0	5	30	50	15	0	0	0
S. tritici . . .	,, ,, . .	0	0	5	10	22	32	29	2
S. nodorum . . .	,, ,, . .	10	30	30	30	0	0	0	0
S. tritici . . .	Potato dextrose agar .	0	0	5	10	10	35	30	10
S. nodorum . . .	Do. .	10	28	32	30	0	0	0	0
S. tritici . . .	Onion agar . . .	0	0	5	10	10	37	30	8
S. local . . .	Wheat-leaf-decoction agar . . .	0	4	28	52	16	0	0	0
S. tritici . . .	Do. .	0	0	6	9	9	30	30	16

(*iv*) *Septation of pycnospores.*—Table VIII gives the degree of septation of pycnospores of the fungi on different media (Plate XVI).

It is clear that the variation in the degree of septation of pycnospores of the various fungi from one medium to another is very slight. A comparison of this Table with Table II, however, reveals that while *S. tritici* has 2-5 septate spores on the host plant, it produces 2-7 septate spores on the culture media on which it forms spores.

S. nodorum has 1-3 septate spores on the host plant and it produces 0-3 septate spores on the culture media.

S. local has remained fairly constant as regards this character.

(*v*) *Size of conidia of* S. local.—As has already been mentioned only *S. local* forms conidia in culture and the other two fungi have not been observed producing this kind of spore. The size of conidia of *S. local* on different culture media is given in Table IX.

TABLE IX

Size of conidia, in microns, of S. local *on different culture media*

Medium	Limits of variation	Average
Oat-meal agar	16·5-38·9×1·5-3·9 . . .	32·7×2·4
Wheat-leaf-decoction agar . .	17·1-37·8×1·5-3·9 . . .	33·1×2·5
Potato dextrose agar . . .	19·2-58·3×1·92-4·2 . . .	38·7×3·6
Onion agar	17·6-53×1·4-2·5 . . .	33·5×1·97
Richard's agar	17·7-44·9×1·8-3·5 . . .	31·7×2·6
Czapek agar	17·6-35·3×1·7-3·5 . . .	27·5×2·6
Brown's agar	17·7-35·3×1·8-3·5 . . .	25·9×2·6
Brown's starch agar . . .	17·7-35·3×2·1-3·5 . . .	25·2×3·1

PLATE XVI

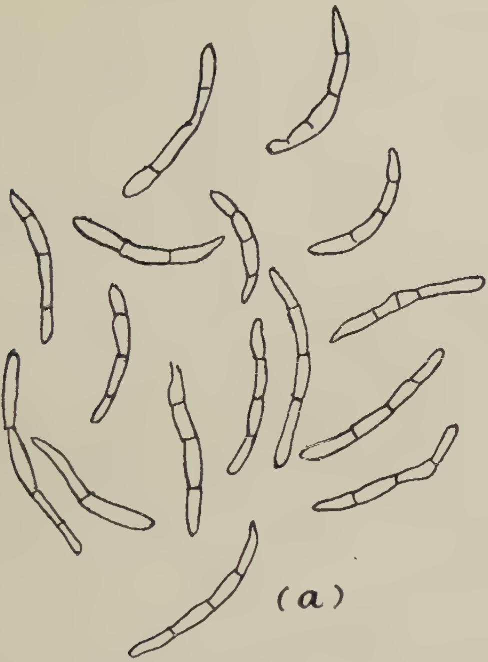


FIG. *a*. Pycnospores of the local species of *Septoria*, from oat-meal agar (after 25 days' growth at 20°C.)

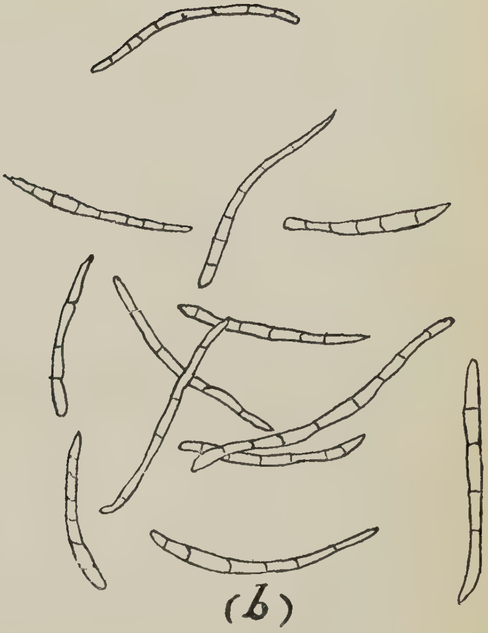


FIG. *b*. Pycnospores of *Septoria tritici* Desm. from oat-meal agar (after 25 days' growth at 20°C.)

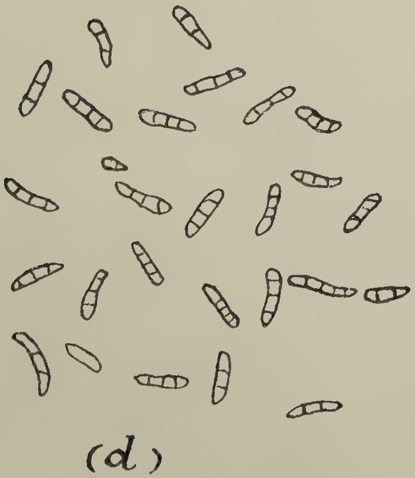


FIG. *d*. Pycnospores of *Septoria nodorum* Berk. from oat-meal agar (after 25 days' growth at 20°C.)

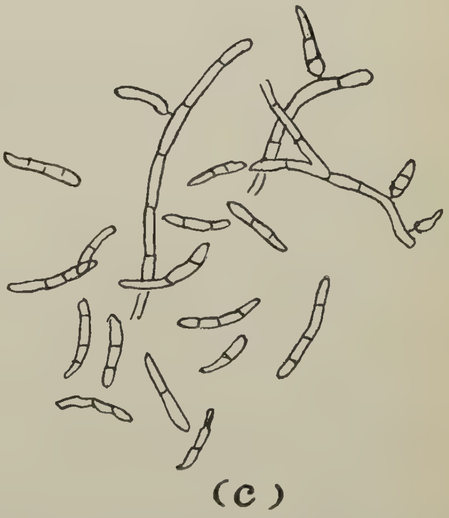
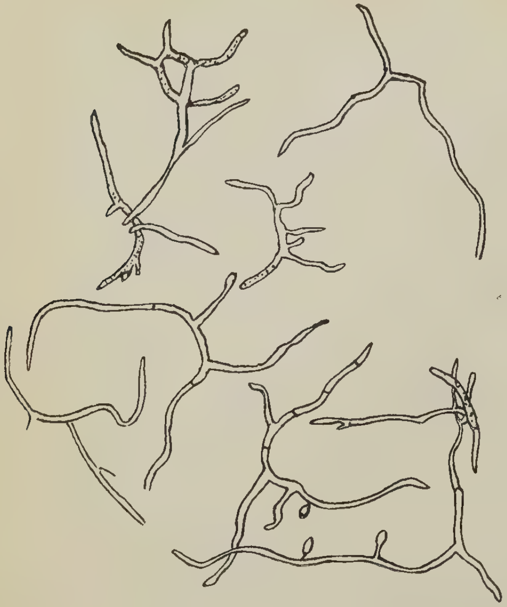
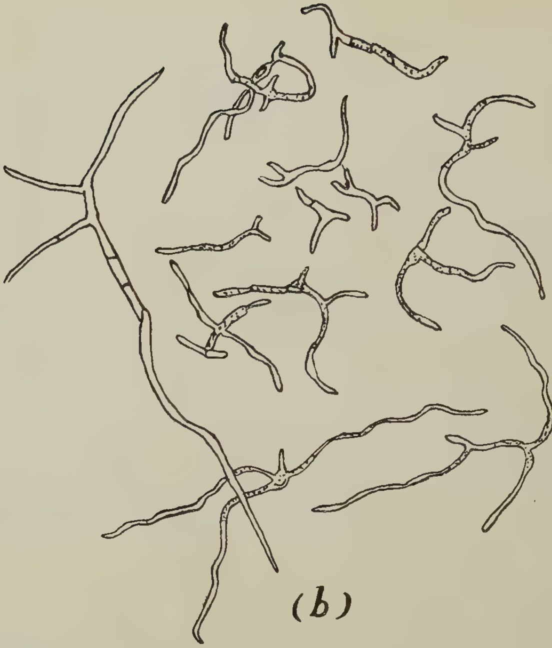


FIG. *c*. Conidia and hyphae of the local species of *Septoria*.



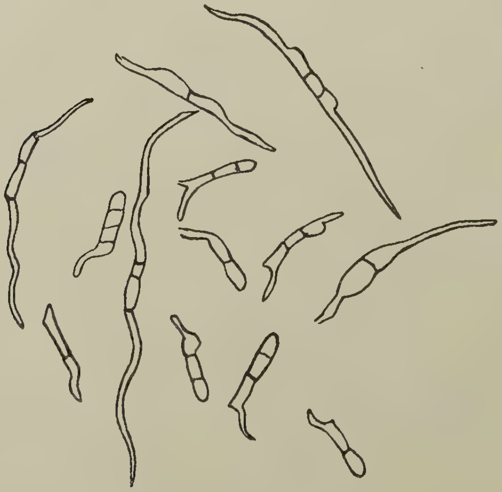
(a)

Fig. *a*. Germinating pycnospores of the local species of *Septoria*, after 24 to 48 hours of sowing in water.



(b)

Fig. *b*. Germinating pycnospores of *Septoria tritici* Desm. after 24 to 48 hours of sowing in water.



(c)

Fig. *c*. Germinating pycnospores of *Septoria nodorum* Berk. after 6 to 12 hours of sowing in water.

The degree of septation of conidia of *S. local* in the different culture media is almost the same (Plate XVI), about twenty-five per cent are continuous, thirty per cent one-septate, thirty per cent two-septate and fifteen per cent three-septate.

(c) *Germination of pycnospores*

(i) *Mode of germination*

S. local.—When pycnospores are sown in drops of water at 20°C. they swell up first and increase both in length and breadth. After about eight to twelve hours some of the spores begin to produce germ-tubes. The germination goes on increasing till, after thirty hours from sowing, it reaches a figure of about ninety to ninety-five per cent. The germ-tubes are produced from both the ends as well as from the middle cells near the septa. At first these are unseptate but after about fourteen hours of their production, septa are laid down. Some of the germ-tubes bear secondary conidia at their ends. These are oval or cylindrical in shape, and at first these are unseptate but soon lay down one or two septa (Plate XVII).

S. tritici.—The method of germination is exactly similar to *S. local* (Plate XVII).

S. nodorum.—The pycnospores of *S. nodorum* begin to germinate after three hours of sowing and there is almost cent per cent germination after twelve hours. The method of germination is the same as described above under *S. local* with the difference that these produce a smaller number of germ-tubes from the middle cells. The germ-tubes also bear a smaller number of secondary conidia (Plate XVII).

(ii) *Effect of different nutrient solutions on germination*

The pycnospores were germinated in 1-3 per cent solutions of potassium nitrate, glucose, potassium phosphate and three different concentrations of wheat-leaf decoction and soil extract. Wheat-leaf decoction was prepared by steaming, for half an hour, sixty grams of green wheat leaves in 300 c. c. of water and soil extract was obtained by suspending fifty grams of soil in 150 c. c. of water and keeping it over night.

The important results obtained are :—

1. The germination of the pycnospores of *S. local* and *S. tritici* is more rapid in wheat-leaf decoction than in any other nutrient solution.

2. No germination of the pycnospores of any of the fungi takes place in potassium phosphate (1-3 per cent). That this is not due to higher hydrogen-ion concentration of the potassium phosphate solution is clear from the results given in Table XI (which is to follow), where it is clear that in glucose solution at *p*H 9·4 good germination takes place in all cases.

(iii) *Effect of temperature on germination*

The optimum temperature for germination of pycnospores of all the three fungi is about 20°C., the maximum between 25 and 30°C. and the minimum below 5°C. The figures illustrative of these results are given in Table X.

TABLE X

Germination of pycnospores of the fungi at different temperatures

Fungus	Temperature (°C.)	Percentage germination after 48 hours	Av. germ-tube length (μ) 48 hours	No. of germ-tubes per spore
S. local	5	73·4	32·4	1·2
S. tritici	5	70·3	25·0	1·6
S. nodorum	5	45·7*	6·7*	1·7*
S. local	10	83·2	51·9	2·4
S. tritici	10	75·0	59·3	2·0
S. nodorum	10	60·3*	16·6*	1·2*
S. local	15	92·7	133·4	7·8
S. tritici	15	86·0	107·8	2·4
S. nodorum	15	97·5*	60·7*	2·1*
S. local	20	95·5	140·5	8·5
S. tritici	20	95·2	166·3	1·6
S. nodorum	20	97·5*	83·9*	2·0*
S. local	25	82·8	126·9	7·2
S. tritici	25	90·0	82·7	1·2
S. nodorum	25	84·0*	55·3*	1·8*
S. local	30	0	0	0
S. tritici	30	0	0	0
S. nodorum	30	0	0	0

* Measured after 12 hours.

(iv) *Effect of hydrogen-ion concentration on germination*

The pycnospores were germinated in two per cent glucose solution having a *p*H range from 2·1 to 9·4. The results are given in Table XI.

It will be seen that the pycnospores of all the fungi can germinate on a very wide range of *p*H. The optimum *p*H for germination of pycnospores of *S. local* is about 6·8, for *S. tritici* about 6·0-6·8 and for *S. nodorum* about 6·8. The spores of all the three fungi do not germinate at *p*H 3·0 and below.

TABLE XI

Germination of pycnospores of the fungi in two per cent glucose solutions of different pH

Fungus	*p*H	Percentage germination	Av. germ-tube length (μ)	No. of germ-tubes per spore
S. local	9·4	82·1	25·8	2·3
S. tritici	9·4	75·8	42·3	1·4
S. nodorum	9·4	82·1*	42·7*	2·1*
S. local	8·6	89·2	42·9	2·6
S. tritici	8·6	80·5	43·5	1·3
S. nodorum	8·6	89·1*	48·0*	2·1*
S. local	7·8	90·7	41·7	2·0
S. tritici	7·8	85·0	52·4	1·8
S. nodorum	7·8	90·1*	77·6*	2·2*
S. local	6·8	97·5	65·2	3·0
S. tritici	6·8	97·3	66·6	1·6
S. nodorum	6·8	97·5*	98·3*	1·8*
S. local	6·0	93·2	48·3	1·6
S. tritici	6·0	93·1	65·8	1·8
S. nodorum	6·0	93·5*	70·0*	2·1*
S. local	5·2	85·2	46·2	1·8
S. tritici	5·2	82·0	51·9	1·4
S. nodorum	5·2	92·8*	75·4*	2·2*
S. local	4·4	75·1	34·7	1·8
S. tritici	4·4	76·5	33·7	1·8
S. nodorum	4·4	94·7*	32·9*	1·6*
S. local	3·8	70·0	23·2	2·1
S. tritici	3·8	70·2	15·2	1·2
S. nodorum	3·8	92·9*	22·3*	2·3*
S. local	3·0	0	0	0
S. tritici		0	0	0
S. nodorum		0	0	0

* Measured after 12 hours.

D. INOCULATION EXPERIMENTS

(*a*) *Inoculation on wheat plants*

Inoculation experiments were conducted on wheat plants in pots as well as in the field. The method followed was as shown below :—

Pycnospores from both the natural specimens and the culture were used. These were suspended in sterile water (about eighty spores to a field under high power) and then sprayed on the plants. An atomizer was used for a small number of plants, and when a large number of plants had to be inoculated, a hand sprayer was used, The potted plants thus inoculated were kept covered for 72-120 hours. In pot experiments when inoculated plants were few, bell jars were used for covering them, but when inoculations were made on a large number of plants either in pots or in field, *sarkanda* screens were erected round the plants and then were covered with *sarkanda* lined with gunny bags. The inoculated plants were kept moist even after the removal of covers.

The results of typical pot experiments are given in Table XII. The results of field experiments were also similar to pot experiments with the difference that percentage and intensity of infection was somewhat less, which is apparently due to the fact that under field conditions it is not possible to maintain as high a humidity in the crop as in the case of plants sown in pots. From the results of all these experiments the following conclusions are drawn :—

(1) All the three fungi are virulent parasites on wheat.
(2) The incubation period of *S. local*, *S. tritici* and *S. nodorum* is about fourteen to sixteen days.
(3) *S. local* and *S. tritici* mainly infect leaves, leaf-sheaths and rarely stems and awns, while *S. nodorum* mainly infects glumes.

TABLE XII

Results of inoculation experiments on Pb 8 A wheat in pots (by pycnospores from culture)

Date of inoculation	Fungus	No. of plants inoculated	Percentage infected				
			Plants	Leaves	Leaf-sheaths	Stem	Ears
1st December 1934	*S. local* .	20	100	83	10	0	No ears on the plants.
	S. tritici .	25	92	78	12	0	Do.
2nd January 1935	*S. local* .	34	100	88	15	0	No ears on the plants.
	S. tritici .	22	100	80	18	0	Do.
	S. nodorum	25	0	0	0	0	Do.
25th March 1935	*S. local* .	40	95	82	35	9	0
	S. tritici .	28	100	71	27	5	0
	S. nodorum	10	50	0	0	0	50

(b) *Inoculation on other plants*

Inoculation on barley (*Hordeum vulgare* Linn.), oats (*Avena sativa* Linn.), *takla* (*Saponaria vacaria* Linn.), *bathu* (*Chenopodium album* Linn.), *lehli*

(*Convolvulus arvensis* Linn.), *pohli* (*Carthamus oxyacantha* Bieb), *maina* (*Medicago denticulata* Willd.) and Sudan grass (*Sorghum Sudanense* Stapf.) have been made several times during the last two years by all the methods above described, but in no case infection took place on any plant.

IV. Discussion of results

A species of *Septoria* which causes the disease of wheat in the Punjab resembles the named species *Septoria tritici* Desm. in all essential respects and is only very slightly different from it in some of the minor characteristics. Both these fungi are highly specialized on wheat and have not been observed attacking any other plant either in nature or under artificial conditions of inoculation. In size the pycnidia and pycnospores of both the fungi on the host are in close agreement with one another. On the host the pycnospores of *S. local* are 1-4 septate and those of *S. tritici* 2-5 septate. Their incubation period is fourteen to sixteen days.

The method of germination of pycnospores of both is the same and both on germination produce secondary conidia in abundance. The cardinal temperature for the germination of pycnospores of both is the same. Both show similar responses to different environmental conditions.

On artificial culture media both grow very slowly, but *Septoria tritici* is somewhat faster growing than *S. local*, its rate of linear growth being about 1½ times that of *S. local*. Further *S. tritici* is characterised by the production of more aerial mycelium than *S. local*. The colour of the colonies of *S. local* is generally black but that of *S. tritici* is white and later changes to pink. In culture the size of pycnidia and pycnospores is approximately the same in both the cases but there is some difference as regards the septation of pycnospores, those of *S. local* being 1-4 septate and those of *S. tritici* being 2-7 septate. *S. tritici* does not produce conidia in culture while *S. local* produces them in abundance.

It is, therefore, clear that these two fungi cannot be distinguished from one another on the host, as they are very similar with regard to their morphology. In artificial culture media also they are morphologically alike and show similar responses to different environmental conditions; and it is only when a careful study of these is made, that they can be differentiated from one another by the following characteristics—

(1) *S. local* grows slower than *S. tritici*, its colony growth being 2/3 of *S. tritici*.

(2) *S. local* is characterised by producing none or very little aerial mycelium in contrast to *S. tritici* which produces a fairly good amount of aerial mycelium.

(3) The colour of the colonies of *S. local* is generally black and that of *S. tritici* white in the beginning, slightly changing to pink later on.

(4) The pycnospores of *S. local* are 1-4 septate and those of *S. tritici* are 2-7 septate.

(5) *S. local* produces conidia in culture, while *S. tritici* has not been observed to produce this kind of spore.

The above characteristics are of little systematic importance as it has been shown by various workers such as Mitra [1931] and others that even greater variation can occur between the saltants of the same species.

It is, therefore, concluded that the *Septoria* species which causes disease of wheat in the Punjab is identical with the named species, *Septoria tritici* Desm. It can, however, be considered a slow-growing strain or saltant of *Septoria tritici* Desm.

Septoria nodorum differs from *Septoria tritici* in all the characteristics which are of systematic importance. Though it is also highly specialised on wheat, it generally attacks the glumes and it is very rarely found on the leaves in nature or when inoculations are made artificially. Its pycnidia are somewhat smaller than those of *Septoria tritici.* The length of its pycnospores on the host as well as in the various artificial culture media is about one-half that of *Septoria tritici* and its pycnospores are continuous to 3-septate whereas those of *S. tritici* are 2-7 septate. Their behaviour under different environmental conditions is, however, similar. Its rate of growth in artificial culture media is about five to seven times that of *Septoria tritici* and it is characterised by the production of copious aerial mycelium.

It is thus evident that *Septoria nodorum* can be easily distinguished from *Septoria tritici* by the smaller size and lower septation of its pycnospores on the host as well as in artificial culture media, its faster rate of growth and production of abundant aerial mycelium in culture. Thus *Septoria nodorum* Berk. is confirmed to be a good species.

V. Summary

1. The following three fungi have been studied :—

(*a*) A species of *Septoria* causing a disease of wheat in the Punjab.

(*b*) *Septoria tritici* Desm.

(*c*) *Septoria nodorum* Berk.

2. The symptoms of the disease and nature of injury caused by the various fungi as observed on naturally or artificially infected plants have been described.

3. A comparative study of the various fungi has been made as regards the following :—

(*a*) morphology on the natural host and on various artificial culture media,

(*b*) response to environmental conditions such as nature of medium, acidity of medium and temperature,

(*c*) manner of spore germination under a variety of conditions.

4. Inoculation experiments have been done on wheat and various other plants in pots as well as in field and the following results have been obtained :—

   (*a*) All the three fungi are highly specialised on wheat.

   (*b*) The species of *Septoria* which occurs locally and *Septoria tritici* mainly attack leaves and leaf-sheaths and rarely stem and awns and *Septoria nodorum* generally causes blotches on the glumes.

5. A discussion of the experimental results in relation to the taxonomy of these forms is given and the following conclusions are reached :—

   (*i*) *Septoria tritici* Desm. and *Septoria nodorum* Berk. are different from one another and are confirmed to be good species.

   (*ii*) The species of *Septoria* causing a disease of wheat in the Punjab is identical with the species *Septoria tritici* Desm. It may, however, be considered a slow-growing strain of *Septoria tritici* Desm.

The authors wish to record their thanks to Dr. E. J. Butler (London) and Messrs. W. W. Mackie (California), G. J. L. Burton (Kenya), H. C. Stenning (New South Wales) and L. R. Tehon (Illinois) for supplying exiccati of either *Septoria tritici* Desm. or *Septoria nodorum* Berk. or both.

REFERENCES

Berkeley, M. J. (1845). *Gard. Chron.* p. 601.

Berlese, A. M. and Pietro Voglino (1886). "Sapro Un Nuovogenere di Funghi Sferopsidei" p. 32 (Extract from *Atti della Societa Veneto-Trentina di Scienze Naturali* **10**, Fasc. 1.) Padova.

Butler, E. J. (1918). 'Fungi and disease in plants', Calcutta.

Clark, W. M. (1923). 'The Determination of Hydrogen-ions.'

Davies, J. J. (1919). *Trans Wisconsin Acad. Sci.* **19**, 671-727.

Desmazieres, J. B. (1842). *Ann. Sci. Nat.* (*Ser. 2*) **17**, 91-118.

——————— (1843). *Ibid.* **19**, 335-373.

Eriksson, Jacob (1891). *Zeitschur. fur Pflanzen-Krankh.* **1**, 27-33.

Grove, W. B. (1916). *Gard Chron.* (*3rd Ser.*) **60**, 194-210.

Kuhn, Julius (1878). *Landw. Verrsuchsst* **21**, 193-194.

Mitra, M. (1931). *Trans. Brit. Myc. Soc.* **16**, 115-127.

Passerini, Giovanni (1877). *Nuovo Giorn. Bot. Ital.* (*Pisa*) **9**, 111.

Rosen, H. R. (1921). *Arkansas Agric. Expt. Stn. Bull.* **175**, 17.

Sydow, H. & P. and Butler, E. J. (1916). *Ann. Myc.* **14**, 177-220.

Voglino, Pietro (1904). *Ann. R. Acad. Agric. Torino* **46**, 259-282.

Weber, G. F. (1922). *Phytopath.* **12**, 449-470 and 537-83.

MOLASSES AS MANURE IN THE UNITED PROVINCES*

BY

H. N. BATHAM, M.A.

Agricultural Chemist to Government, United Provinces

R. L. SETHI, M.A., B.Sc.

Economic Botanist to Government, United Provinces

AND

L. S. NIGAM, L.Ag.

Research Assistant to the Agricultural Chemist

(Received for publication on 10th November 1936)

INTRODUCTION

In sugar-producing countries the disposal of molasses has long been a problem of vital importance. The molasses has been put to many varied uses in other countries, such as the production of alcohol, organic acids, glycerine, road-making material, cattle feed, manure, etc. With the rapid growth of the sugar industry during the last few years the disposal of molasses has also become acute in India. Under present conditions there is practically no sale of molasses, though a small quantity of the material is used for the production of alcohol and other sundry purposes. Attention naturally turns to the discovery of some other use, which will consume the major portion of this bye-product, and one such use can be as a fertiliser.

The Department of Agriculture of the United Provinces started the investigation of the use of molasses as manure from the year 1932, first in pots and later on a field-scale. Dhar and his colleagues [1934-35] also began their researches with the use of molasses as manure in these provinces.

This paper deals with the investigations carried out by the Agricultural Department in the United Provinces and summarises experience gained by other countries.

In Mauritius [Verret and Doty, 1934] molasses has formed an important item in fertiliser practice since 1860 and planters insist on a return by the factory of a certain percentage of the molasses from the cane delivered. Molasses is applied there a month or fortnight before planting; and to ratoons on both sides of the stools immediately after the harvest. The work done in Mauritius notes the depressing effect on nitrification referred to as partial sterilisation with the growth of moulds and torulae followed by a resumption of nitrification at a greater rate.

---

*Read at the Annual Convention of Sugar Technologists' Association of India, 1936.

The result of fermentation is to liberate some of the unavailable reserve of plant food and also to improve the physical condition of the soil.

There are considerable differences of practice with regard to the application and quantity applied. Of particular interest is the general agreement as to the depressing effect on the juices with the exception noted by the Oahu and Hawaiian Commercial Sugar Companies that suitable amounts applied at the correct time have no effect on the juice. The Pioneer Mill Company notes that the increased yield of cane more than offsets the poorer juice, while increased vigour and colour of plants and the improved physical condition of the soil are beneficial effects, which persist for several crops.

Owing to the denitrifying effect it is best to make a single large application to ratoons. The optimum amount appears to be five tons per acre and the best time for application is with the last irrigation water after the harvest. The evidence indicates that the juices of the old canes are not affected and the denitrifying effect has passed before the new crop starts to grow. For plant-canes twenty tons or more is usually applied before ploughing.

The effects of fertilisation with molasses on the production of sugar varies. Generally there is a gain, often a large gain, but generally also the juices are poorer. On soils of a heavy clay type the leaves turned yellow and in several cases the plants died. This occurred in poorly drained soils where fermentation took place under anaerobic conditions and the suggestion is made that in well-drained soils with a high aluminium content the application of lime with molasses is desirable.

Cross [1911] calculated the value of molasses as fertiliser and showed it to produce on an average 3 mg. nitrogen per gram of sugar used. The molasses furnished carbohydrates to the nitrogen-assimilating bacteria, which in consequence became more numerous and more active, assimilating nitrogen in proportion to the amount of sugar added. Kuyper [1926] showed that on certain types of heavy clay soil of bad quality the addition of molasses exerts a favourable effect. Heck [1931] found that in gravelly soil, under laboratory conditions, molasses added at the rate of ten tons per acre was rapidly decomposed. This was accompanied by a large increase in soil organisms and a definite rise in temperature and only one quarter of the carbon in sugar re-appeared in the carbonic acid gas given off, the rest apparently being locked up in the micro-organic material. He further points out that a nitrogen relationship exists in the decomposition of molasses in the soil. If sodium nitrate is present in sufficient quantities, forty pounds of nitrate is removed from the soil and apparently built up into micro-organic material for every ton of molasses decomposed, and that no loss is occasioned by this change in the form of nitrogen, in that it is locked up in micro-organisms and will be available for the plant at a later stage. He also found that yeast fermentation is stimulated by molasses application to the soil and since, as he observes, the more fertile a soil may be the more active biologically it will be found, there can be little doubt as to the beneficial role, which molasses plays.

Recently a somewhat voluminous report has been issued from Java [Melaselumestig, 1932] and reviews all the experimental work done in that island on the manurial use of molasses. It says molasses may be applied either diluted or undiluted at one of three seasons, before the rice crop preceding cane, before planting the cane and during the growth of the latter. In the first case there is the effect of the application on the rice crop to be taken into consideration. In twenty-eight experiments covering this aspect an average increase of yield of forty-three per cent was obtained with the evidence that the yield increased with increasing amounts of molasses. In none of these trials was a reduced yield obtained. The residual effect on the succeeding crop was not as great ; in twenty-two out of twenty-eight cases an increased yield of sugar was obtained with a maximum recorded increase of thirty-two per cent and a maximum decrease of nine per cent.

The effect of molasses, when applied before planting the cane crop, has been tested in a very large number of experiments. Here a difference in the effect is found to be associated with the class of soil as also with the water supply. While in the whole series fifty per cent of the experiments showed an increase from the use of molasses and fifteen per cent a decrease, the corresponding results on sandy soil were seventy-three per cent and three per cent respectively and on heavy clay forty-nine per cent and twenty per cent. The general conclusion drawn is that the more marked effect obtained on light soil is due to the lesser fertility of the soils on which the experiments happened to be conducted. The effect of molasses on sugar production is the reverse and the average decrease in all experiments was 0·19 per cent. This reduction in the sugar content was greatest on light soils and was emphasised by increasing the quantity of molasses applied. Where irrigation was reduced, the increase in yield was relatively large, indicating that molasses increases the retentive capacity of the soil. Though found beneficial generally, the effect of the application of molasses to the growing crop is less marked than when the application is made before planting and the reduction in sugar content is more marked. The best results were obtained when molasses was applied to the ridges and, further, fresh molasses proved to be less beneficial than one-year-old molasses.

From a comparison of the advantages and disadvantages of the various methods adopted for the application of molasses the main conclusions drawn are as follows :—

(1) The application of molasses with irrigation water in a quantity equivalent to one blik per guel (1 blik = 18 litres and 1,000 guel = 1 hectare) whether before the preceding rice crop or before cane crop, is one of the cheapest methods of application, but in both cases the application must be made at least three weeks before the planting of the crop.

(2) In the above case the molasses is applied to the land before trenching. It may also be applied in the trench before planting. It must be applied at least three weeks before planting, but at the rate of one blik per guel.

(3) The application may also be made after planting either diluted or undiluted molasses on the ridges or in the trenches in very diluted form (approximately 0·1 per cent) with irrigation water and of these the former is preferable. Application of undiluted or slightly diluted molasses to the growing crop in the trenches has nothing to recommend it.

The summary of experience in different countries is that, for successful results, aerobic conditions are desirable and that the value of an application will be doubtful, where soil and weather conditions render such conditions difficult to attain. Also, seeing that a depressing effect in the first instance is inevitable, the time of application should be chosen before planting or when the plant is entering a dormant condition.

Viswanath [1933-34] carried out at Coimbatore experiments of a preliminary nature to study the bio-chemical changes taking place when molasses are added to puddled paddy soils drained as well as undrained and the results showed :—

(1) an enormous increase in bacterial population in about six days after the application of molasses, the increase being greater in the drained than in the undrained soil;

(2) the absence of yeast and algae in the molasses-treated soils at any period under observation;

(3) a change in physical texture of the soil in the molasses plots which puffed up and became soft to the touch, due probably to the production of organic acids, evolution of gases and the consequent rise in temperature; and

(4) the complete absence of the added sugar in the molasses pots at the end of even two days after the addition of molasses.

The analyses of the gases evolved during the period of vigorous fermentation of molasses lasting for about a week showed a large percentage of carbon dioxide, some hydrogen and very little methane. No nitrogen was detected at any stage of fermentation. Estimation of various bye-products, acetic, butyric and lactic acids, made periodically at the end of three, eight and fifteen days in molasses-treated soils under swampy conditions, showed a rapid increase in lactic acid up to ten days followed by a gradual decrease in the same, indicating thereby utilisation of the acid itself by the micro-organisms as a source of energy.

Dhar and his co-workers [1934-35] have studied some of the conditions necessary for nitrogen-fixation, ammonification and nitrification in soils, particularly when molasses is added to it.

Micro-organisms are believed to play a very large part in soil fertility, and nitrogen-fixation, ammonification and nitrification are carried on in soils by different kinds of bacteria and some of them are said to have been isolated from soils by different methods and by only a few scientists. Though a lot of work has been done in soil bacteriology still quite a considerable amount of doubt exists about the actual significance of the so-called active bacteria, particularly ammonifiers and nitrifiers, of culture media in relation to their actual functions in the soil. On the other hand these bacteria change their appearance so easily with slight variations in conditions and many of them can effect so wide a range of chemical reactions that it is difficult to characterise them exactly. They require carbohydrates and proteins as foods and energy material. It is also known that the production of the same kind of chemical reaction is brought about by not only a particular form of bacteria but by other forms as well. Berthelot [1885] found that certain organic compounds could absorb free nitrogen under the influence of silent electric discharges. Batham and Nigam [1930] have shown that the greatest accumulation of nitrate takes place during the summer months and the least during winter ones and that there seems to exist a close relationship between the fluctuations of nitric nitrogen contents of soils and different seasons of the year, that is, solar activity.

Dhar and his co-workers [1934-35] show that the fixation of nitrogen in the soil by the addition of sugar is helped by light and may take place in the absence of azotobacter. According to them the first product in nitrogen fixation is nitrate which is further reduced to ammonia. They summarise their work thus :—

(1) When cane sugar is added to sterilised and unsterilised soils and exposed to light and air, there is appreciable increase in the ammoniacal nitrogen content, with unsterilised soil the ammoniacal nitrogen is nine times greater than that originally present in the soil.

The amount of ammonia in presence of light is always greater than that in the dark.

(2) By the addition of molasses to soils which have been properly aerated the ammoniacal nitrogen is three times greater than that originally present in the soil, when 3,600 kg. of molasses are added per acre of land. When the aeration of the soil is insufficient the increase of ammonia is less and soil becomes acidic.

(3) Under completely sterilised conditions when cane sugar is mixed with soil there is also an increase in the ammoniacal nitrogen. In one experiment the total available nitrogen (ammoniacal and nitrate nitrogen) in the soil was 0·0005, but after exposure to sunlight in a flask with cane sugar it became 0·0098. The conclusion has been drawn that just as bacteria can fix nitrogen in the soil in presence of energy-rich compounds, similarly even in the absence of bacteria the photo-oxidation of the energy-rich compounds leads to the fixation of nitrogen.

It appears, therefore, that in tropical countries in ordinary soils, the fixation of nitrogen by the addition of an energy-rich compound may be partially bacterial and partially photo-chemical. The oxidation of energy-rich organic compounds by air either by induction or light absorption or bacterial action, causes the fixation of atmospheric nitrogen in the soil. Moreover this condition is facilitated by increase of temperature and light and hence there is a great possibility for the utilisation of molasses in India as a manure in increasing the soil nitrogen which is her crying need.

MANURIAL INGREDIENTS IN MOLASSES

The use of molasses as a manure necessitates a knowledge of the manurial ingredients contained in it. Consequently in several samples of molasses used at different farms essential plant-foods were determined and the results are given in Table I.

There is a wide range of variation from five to twelve per cent in the ash contents of molasses. Molasses numbered 3, 4 and 5 are from *gur* refineries and contain more ash than those from cane factories. They are all poor in nitrogen but rich in potash. The percentage of nitrogen varies between 0·23 and 0·44 and that of potash between 1·48 and 2·84. The percentage of phosphoric acid is slightly greater than that of nitrogen and varies between 0·27 and 0·67. Cane molasses contains about fifty-five per cent sugars and twenty per cent water.

TABLE I

Percentage of essential plant foods found in molasses

No.	Description of samples of molasses	Ash in molasses (per cent)	Nitrogen molasses (per cent)	K_2O molasses (per cent)	P_2O_5 molasses (per cent)
1	Obtained from Carew & Co., Ltd., Shahjahanpur, through Sugarcane Research Farm .	5·25	0·24	2·53	0·35
2	Ditto . .	6·56	0·24	2·84	0·49
3	Obtained from Union Indian Sugar Works, Ltd., Cawnpore, through Cawnpore Research Farm	12·06	0·23	2·79	0·21
4	Obtained from Cawnpore Sugar Works, Ltd., Cawnpore .	9·87	0·25	2·81	0·29
5	Obtained through the Government Experimental Farm, Gorakhpur	10·49	0·43	1·48	0·67
6	Obtained through Government Farm, Meerut . . .	..	0·44	2·25	0·65

NOTE.—Molasses used at Sugarcane Research Farm, Shahjahanpur, contained 0·74 per cent lime.

POT-CULTURE EXPERIMENTS

Pot-culture experiments were started at Cawnpore in July 1932 with an air-dried soil in fifteen earthen pots. They were divided into five sets, each consisting of three pots. The soils of the first set were treated with water sufficient to bring the moisture content up to the optimum, which was found to be twenty per cent. The soils of the second, third, fourth and fifth sets were also given the same amount of water with this difference that the soil of the second set was mixed with molasses at the rate of six tons per acre, that of the third set contained a mixture of fertilisers consisting of ammonium sulphate, potassium sulphate and sodium phosphate and containing nitrogen, potassium and phosphorus in the same proportions and weights as contained in six tons of molasses. The water of the fourth set contained potassium sulphate and sodium phosphate, the proportions and weights of potassium and phosphorus being the same as in the third set. The fifth set had only ammonium sulphate with the same quantity of nitrogen as in the third and fourth sets. Two pots of each set had crops and the third was kept fallow. Six crops have been taken from them, three *Kharif* and three *Rabi*. For *Kharif*, *kakun* (*Panicum italicum*) was selected and for *Rabi*, wheat (*Triticum sativum*). About the middle of August another set of three pots was prepared having the same amount of soil and water but the quantity of molasses was doubled, *i.e.*, twelve tons per acre. The optimum moisture content of each pot was kept up by weighing each one every week and adding water to make up its loss due to evaporation and transpiration.

The yields of grains and straws of the six crops obtained from the two pots of each treatment are given in Table II. The low yields of the first two crops from all pots were due to the fact that they were sown about a month later than their usual time. Among the low yields, the lowest were obtained from molasses-treated pots. This confirms the views of Peck [1912] and other workers that in all cases the effect of the application of molasses was to depress the rate of nitrification particularly in the early stages, recovery taking place subsequently on account of partial sterilisation. The yields of the crops of 1933-34 and 1934-35 from molasses-treated pots have decidedly improved not only upon the yields of the previous crops obtained from the same pots but also upon the yields from other pots treated otherwise. The total yields of grain and straw of three *kakun* crops from molasses-treated pots are greater than those from no-manure and ammonium sulphate pots.

TABLE II

Yield of grain and straw of each crop from two pots of each treatment

Treatment	Yield of *kakun* in grams								Yield of wheat in grams							
	1932-33		1933-34		1934-35		Total		1932-33		1933-34		1934-35		Total	
	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw
1. No manure	0·398	0·832	7·56	12·78	9·20	15·67	17·158	29·282	3·00	6·34	5·64	7·35	10·30	15·20	18·94	28·89
2. Molasses 6 tons per acre . .	0·223	0·723	8·33	16·08	10·57	17·89	19·123	34·893	2·80	6·16	5·34	7·20	10·78	14·67	18·92	28·03
3. Composite of fertilisers containing nitrogen, potash and phosphorus as contained in 6 tons molasses.	0·374	1·210	11·89	19·26	12·06	19·75	24·324	40·220	3·06	6·26	6·20	6·75	10·65	15·05	18·91	28·06
4. Composite of fertiliser containing potash and phosphorus as contained in 6 tons molasses.	0·278	0·991	11·91	18·90	11·98	20·00	23·168	39·891	3·31	7·59	6·15	7·17	13·62	17·18	23·08	31·94
5. Ammonium sulphate containing nitrogen as in 6 tons molasses.	0·285	0·502	8·71	14·76	10·05	16·43	19·045	31·692	2·16	5·66	5·74	7·61	10·89	14·37	18·79	27·64
6. Molasses 12 tons per acre . .	...	...	...	...	...	...	...	...	2·09	6·67	6·44	7·66	13·01	17·89	21·54	32·22

The total yield of wheat straw is highest from pots treated with twelve tons molasses and the yield of grain is also next to the highest. Leaving aside the case of pots treated with potash and phosphatic manures, the total yields of wheat grain and straw, obtained from pots having six tons of molasses, do not differ much from those obtained from the remaining pots. Thus the results of crop yields obtained from molasses treatments indicate the possibility of utilising molasses as manure for both *Kharif* and *Rabi* crops.

PLANT CANE

The field-scale experiments were first started on the sugarcane crop of 1933-34 at Cawnpore and Shahjahanpur according to the randomised block method. The area of each plot was 1/40th of an acre. At Cawnpore each treatment had five replicates and at Shahjahanpur four. At the former molasses was compared with other fertilisers. Molasses was applied at the rate of six and twelve tons per acre two months before planting and two months after planting the crop. Two mixtures of fertilisers were prepared, consisting of ammonium sulphate, potassium sulphate and bone superphosphate. They were applied simultaneously with molasses and their quantities were regulated by the proportions and weights of nitrogen, phosphorus and potassium as contained in six and twelve tons molasses. The molasses and fertilisers were applied to the trenches, which were then flooded with water and trodden by men to dissolve it and when sufficiently dry the trenches were hoed. After three similar waterings and hoeings at intervals of about a fortnight each to ensure thorough mixing of molasses with the soils and to keep it sufficiently aerated, cane setts of Co 213 were planted in all plots in the beginning of March 1933. The cane crop of 1934-35 was also sown at about the same time of the year, but during the period of its germination there was a stoppage of the supply of canal water for a sufficiently long time to create favourable conditions in several of the cane-fields of the Cawnpore Research Farm for white ants to damage the seedlings badly. Consequently countings of germinating setts were carried out and the percentage of germination calculated to get an idea, if possible, of the comparative values of different treatments. After the harvest of the cane crops Guinea grass was grown on the cane field of 1933-34 and maize on that of 1934-35. The yields of the two cane crops along with the sucrose and glucose contents of their juices as well as yields of the two subsequent fodder crops are given in Table III.

TABLE III

Comparative effect of molasses and other fertilisers on the composition and yield of sugarcane Co 213 during 1933-34 and 1934-35 and their residual effect on the subsequent crops at Cawnpore, Co 312 at Muzaffarnagar and Co 213 at Bahraich

Station and year	Time of application	Name and quantity of manure applied	Juice expressed per 100 cane	Sucrose in juice per cent	Glucose in juice per cent	Purity	Actual yield of cane per plot in lbs.	Germination of setts per cent	Calculated yield of cane per acre in lbs.	Calculated yield of sucrose per acre in lbs.	Yield of subsequent crop per acre in lbs.
Cawnpore 1933-34.	Two months before sowing cane crop.	Molasses 6 tons per acre . .	71·96	15·41	0·53	85·89	1,852	...	74,080	8,215	18,184
		Molasses 12 tons per acre .	72·26	15·73	0·43	86·65	1,863	...	74,520	8,473	18,912
		Fertilisers equivalent to 6 tons molasses per acre.	70·77	14·91	0·85	85·78	1,973	...	78,920	8,326	18,404
		Fertilisers equivalent to 12 tons molasses per acre.	71·62	14·59	0·96	85·35	1,949	...	77,960	8,149	18,776
		No manure	72·42	15·37	0·86	85·63	1,858	...	74,320	8,272	17,952
	Two months after sowing cane crop.	Molasses 6 tons per acre . .	72·79	15·35	0·52	87·13	1,790	...	71,600	7,998	17,532
		Molasses 12 tons per acre .	74·80	15·20	0·38	86·70	1,698	...	67,920	7,722	20,132
		Fertilisers equivalent to 6 tons molasses per acre.	76·33	15·47	0·69	86·60	1,864	...	74,560	8,805	17,676
		Fertilisers equivalent to 12 tons molasses per acre.	74·93	15·60	0·58	87·72	1,947	...	77,880	9,104	18,572
		No manure	74·14	15·61	0·59	88·42	1,838	...	73,520	8,506	17,020

Cawnpore 1934-35.	Two months before sowing cane crop.	Molasses 6 tons per acre . .	53·60	16·32	0·27	85·89	1,145	52·6	70,520	6,354	9,735
		Molasses 12 tons per acre .	56·40	17·23	0·29	87·50	1,174	51·2	74,292	7,221	9,603
		Fertilisers equivalent to 6 tons molasses per acre.	57·70	17·17	0·39	87·58	1,812	73·8	79,564	7,884	9,735
		Fertilisers equivalent to 12 tons molasses per acre	60·40	16·33	0·51	86·27	1,922	78·4	79,440	7,843	10,962
		No manure	45·37	17·39	0·34	87·92	1,236	62·2	64,384	5,080	8,580
	Two months after sowing cane crop.	Molasses 6 tons per acre . .	55·70	15·87	0·52	84·75	1,692	76·2	71,952	6,361	13,799
		Molasses 12 tons per acre .	57·60	15·92	0·42	84·62	1,441	64·4	72,480	6,646	16,632
		Fertilisers equivalent to 6 tons molasses per acre.	58·70	15·85	0·52	85·16	1,608	76·6	68,145	6,337	13,325
		Fertilisers equivalent to 12 tons molasses per acre.	56·90	15·35	0·58	84·04	1,572	74·0	68,804	6,007	15,592
		No manure	54·40	16·21	0·46	85·56	1,982	81·0	79,272	6,992	13,563
Muzaffarnagar 1934-35.	Two months before sowing cane crop.	Molasses 200 maunds per acre .	78·94	12·12	...	85·11	2,759	...	110,185	10,544	...
		Farmyard manure 200 maunds per acre.	70·90	12·34	...	84·29	2,633	...	105,344	9,218	...
		Neem cake 200 maunds per acre	70·65	...	...	...	...	...	...	...	...
		Town sweepings 50 cart loads per acre.	72·24	13·01	...	86·56	2,659	...	106,354	9,997	...
Bahraich 1934-35.	Three months before sowing.	Molasses 6 tons per acre . .	...	...	...	...	1,965	...	78,600	...	...
		Molasses 12 tons per acre .	...	...	...	...	1,958	...	78,320	...	...
	One month and half before sowing.	Cow-dung 300 maunds *plus* castor cake 12 maunds per acre.	...	...	...	...	2,430	...	97,200	...	...
		No manure	...	...	...	...	1,845	...	73,800	...	...

NOTE.—The subsequent crop at Cawnpore after sugarcane of 1933-34 was Guinea grass and that after sugarcane of 1934-35 was maize.

At Muzaffarnagar the effect of 200 maunds of molasses containing 25·75 lbs. nitrogen per acre was examined on Co 312 and compared with those of 200 maunds of farmyard manure containing 70·75 lbs. nitrogen and fifty maunds of *neem* cake containing the same amount of nitrogen as contained in the farmyard manure. Each treatment was replicated five times.

At Bahraich the effect of different doses of molasses on Co 213 was compared with that of cow-dung manure applied a month and a half before planting, but no chemical analysis was done. There were four replicas for each treatment. The results obtained at Muzaffarnagar and Bahraich are also included in Table III.

At Shahjahanpur the same two factors, *i.e.*, the quantity of molasses and the time of application, were varied as at Cawnpore, but no comparison was made with any other manure or fertilizer except with the controls having no manure. During 1933-34 three quantities of molasses, thirty maunds, sixty maunds and ninety maunds per acre were applied at three different times, two months before sowing the cane crop, at the sowing time and at the tillering time. As, out of all the treatments in the first year, the greatest yield of cane and the richest juice were obtained from ninety maunds molasses applied two months before sowing ; therefore for the experiments of 1934-35 ninety maunds of molasses was taken as the minimum dose, the other two doses being 180 maunds and 270 maunds of molasses per acre and the times of application were kept the same as in previous year. During 1933-34 variety Co 213 and during 1934-35 variety Co 300 were sown and the number of replicas was four for each treatment. Here as well as at Muzaffarnagar the molasses used was one year old. The data obtained are given in Table IV.

The effect of variations in time of application of molasses was noticed in the depression produced for a short while on the growth of young plants treated with molasses two months after planting. Though this depression in appearance gradually disappeared during rainy season, still it remained and lowered the yields of both cane and sucrose per acre as can be seen from the figures.

TABLE IV

Effect of molasses in different doses given at different times on the yield of sugarcane variety Co 331 during 1933-34 and Co 300 during 1934-35 at Shahjahanpur

Year	Time of application	Treatment	Juice expressed per 100 cane	Sucrose in juice per cent	Glucose in juice per cent	Purity	Yield of cane per acre in lbs.	Yield of sucrose per acre in lbs.
1933-34 .	Two months before sowing cane crop.	30 mds. molasses .	..	11·53	1·61	75·35	146,920	
	Do. .	60 ,, ,, .	..	11·58	1·66	75·11	144,080	
	Do. .	90 ,, ,, .	..	12·17	1·37	78·11	148,640	
	At sowing time.	30 ,, ,, .	..	11·08	1·71	74·94	138,280	
	Do. .	60 ,, ,, .	..	11·35	1·71	75·52	141,420	
	Do. .	90 ,, ,, .	..	11·38	1·66	74·44	143,628	
	At tillering time.	30 ,, ,, .	..	11·72	1·63	76·21	142,960	
	Do. .	60 ,, ,, .	..	11·71	1·65	76·32	144,600	
	Do. .	90 ,, ,, .	..	11·51	1·68	75·71	146,800	
		No manure .	..	12·06	1·57	77·10	140,800	
1934-35 .	Two months before sowing.	90 mds. molasses .	66·9	16·56	0·21	92·15	53,758	5,956·4
	Do. .	180 ,, ,, .	67·7	16·38	0·20	91·66	63,289	7,019·2
	Do. .	270 ,, ,, .	68·9	16·06	0·21	91·92	67,406	7,455·1
	At sowing time.	90 ,, ,, .	65·5	16·45	0·23	93·10	47,339	5,098·4
		180 ,, ,, .	66·5	16·57	0·20	93·24	50,121	5,523·3
		270 ,, ,, .	66·4	16·50	0·20	92·85	55,190	6,048·8
	At tillering time.	90 ,, ,, .	65·4	17·24	0·16	92·79	51,554	5,810·1
		180 ,, ,, .	62·7	17·28	0·16	92·51	46,943	5,083·9
		270 ,, ,, .	63·2	16·85	0·20	91·18	45,923	4,891·0
		No manure .	66·4	15·90	0·29	90·50	49,462	5,223·2

The figures of the percentage of sucrose in the juice of Table III do not show in the year 1933-34 any reduction in the sucrose content of juice by the application of molasses two months before sowing, on the other hand the percentage of glucose in the juice of canes treated with molasses is generally found less than that in the juice of canes treated otherwise. The application of twelve tons molasses per acre has always given better yields of cane and sucrose than that of six tons and compares favourably with that of fertilisers in the production of sucrose per acre. The differences among the yields of canes as well as sucrose per acre obtained from different treatments are statistically quite insignificant. The reason for this appears to be that on account of green manuring of the cane field with sunn-hemp the soil had already sufficient plant food to grow a good crop of canes. Further additions of molasses and fertilisers must have been over and above the requirements of the crop and therefore could not probably produce any significant difference in the yields.

Looking at the column of the yields of subsequent crops it is seen that the residual effect of twelve tons molasses is on the whole best and the increase in the outturns of the subsequent crops obtained from twelve tons molasses over those from the controls is also in some cases statistically significant.

The data procured from Bahraich go to show that the tonnage of canes from molasses treatment is not so high as that from cow-dung *plus* castor cake, but is superior to that from the controls. At Muẓaffarnagar molasses has given the highest yield of cane though this increase is statistically insignificant.

Table IV gives the results of Shahjahanpur experiment. From the columns of the percentage of sucrose and glucose in the juice of the year 1933-34 it is seen that the juice obtained from all the treatments is poorer in sucrose and richer in glucose than that from no-manure except that from ninety maunds molasses applied two months before planting, which has given the richest juice and the highest tonnage. Leaving aside the case of thirty maunds molasses applied at the sowing time the tonnage from all treatments is higher than that from no-manure.

During the year 1934-35 all the molasses treatments have given greater percentage of sucrose and less percentage of glucose in the juice than no-manure. Considering the outturns of cane and sucrose per acre it appears that though the application of molasses is generally beneficial still its effect when applied to a growing crop is less marked than when it is applied before planting. It is observable that the outturn of cane as well as of sucrose per acre increased with the increase in the quantity of molasses applied two months before planting and at the planting time, while in the case of molasses applied at the tillering time the yield of both per acre decreased with the increase in the quantity of molasses applied. The variations in the increase of yields with the increasing quantities of molasses applied have also been observed by other workers and consequently the quantities of molasses applied in other countries go up to forty tons per acre. It is interesting to note that the high yields of the crop obtained from 180 maunds and 270 maunds of molasses applied two months before sowing are statistically significant over that of the control plots. The difference in the outturn of canes when molasses is applied at different times is due to certain biological changes occurring in the soil by the addition of molasses.

Tempany and others [1924] have found that the first result of molasses application appears to be the elimination of active nitrifying organisms, but those responsible for the reversion of nitrates to organic nitrogen do not appear to be affected to the same extent and may even be stimulated in the form of torulae and moulds, the result being, after a short time, that all the nitrates existing in the soil become reverted to the insoluble form of organic nitrogen. Subsequently this class of organisms becomes reduced, possibly owing to the removal of nitrates and ammonia. Afterwards when nitrifying organisms once more begin to populate the soil, they produce nitrates at enhanced rates, the result being practically the same as in other cases of partial sterilisation.

The low yields of cane and sugar obtained from molasses applied at the sowing time and particularly at the tillering time is due to the stoppage of the supply of available nitrogen to the growing plant at the time when it is mostly needed for their development. The cause of the beneficial results obtained from molasses applied two months before sowing appears to be that by the time the plant begins to take its food from the soil the reversion of available nitrogen into unavailable form becomes reduced and the nitrogen fixation, ammonification and nitrification begin to intensify in the soil, either due to bacterial or photochemical reactions, producing available nitrogen at enhanced rates and supplying it to the growing plants.

RATOON CROPS

The effect of molasses on ratoon canes of Co 213 was investigated in comparison with that of castor cake alone and castor cake with bone superphosphate, at Gorakhpur Experimental Farm during the years 1933-34 and 1934-35, and at Cawnpore Research Farm during the year 1934-35. At both places molasses was applied at the rate of twelve tons, castor cake twenty maunds and superphosphate three maunds per acre and the results obtained are given in Table V.

TABLE V

Results of molasses as manure with ratoon canes of Co 213

Year	Station	Treatment	Juice per 100 cane	Sucrose in juice per cent	Glucose in juice per cent	Purity	Yield of cane per acre in lbs.	Yield of sucrose per acre in lbs.	Percentage yield of cane	Percentage yield of sucrose
1933-34 .	Gorakhpur .	No manure	56·31	15·76	0·67	90·50	48,052	4,262	100·00	100·00
,, .	,, .	Castor cake alone	58·16	13·71	1·21	85·76	60,608	4,830	126·13	112·37
,, .	,, .	Castor cake with bone superphosphate.	57·20	13·83	1·22	86·00	61,180	4,839	127·32	112·28
,, .	,, .	Molasses	57·50	13·15	0·89	83·94	64,252	4,857	133·71	113·59
1934-35 .	,, .	No manure	64·10	16·01	0·20	88·57	33,110	3,397	100·00	100·00
,, .	,, .	Castor cake alone	64·30	16·75	0·24	87·86	48,653	5,240	146·94	145·47
,, .	,, .	Castor cake with bone superphosphate.	63·40	16·84	0·26	86·09	50,666	5,411	153·02	150·22
,, .	,, .	Molasses	64·40	17·11	0·20	88·84	62,007	6,827	187·27	189·70
,, .	Cawnpore .	No manure	61·34	17·49	0·17	88·16	58,368	6,263	100·00	100·00
,, .	,, .	Castor cake alone	62·47	17·56	0·36	87·77	65,292	7,182	111·86	117·63
,, .	,, .	Castor cake with bone superphosphate.	62·75	16·28	0·18	88·24	77,264	7,889	132·37	127·35
,, .	,, .	Molasses	63·19	16·39	0·15	86·98	79,568	8,243	136·32	134·32

Considering the percentage of sucrose in juice given in Table V it is seen that out of three cases only in one case molasses-treated canes have given richer juice in sucrose, and in others poorer juice than the untreated or otherwise treated ones. This is in agreement with the findings in Mauritius, where juices are generally found poorer in sucrose from molasses treatment but the observation by the Oahu and Hawaiian Commercial and Sugar Companies and the Pioneer Mill Co. that the suitable amounts applied at correct time have no effect on the juice has still to be worked out for the United Provinces. In all cases molasses has given the highest yield of ratoon canes and the difference between the yields of canes from molasses treatments and those from no-manure are statistically significant both at Gorakhpur and Cawnpore.

WHEAT CROP

Experiments with wheat were carried out at the Cawnpore Research Farm during the years 1933-34 and 1934-35 according to the randomised block method on soils, which were poorest in fertility on the farm. In 1933-34 the manures used were molasses alone, molasses with lime and cow-dung manure and in 1934-35 compost was substituted in place of cow-dung manure. Molasses and cow-dung were applied at the rate of 2·5 tons and lime 0·5 ton per acre. The time of application was changed. The first application of molasses alone and molasses with lime was made in July, the second in August and cow-dung or compost manure was applied in September. There were five replicates for each treatment at Cawnpore and four at Gorakhpur, where the effect of molasses was not compared with any other fertiliser but only with controls receiving no manure. At Cawnpore the residual effects of different manures applied to wheat fields were also observed on the subsequent sunn-hemp crops, which were sown two months after harvesting wheat. The data obtained are given in Table VI and go to show that though molasses does not give as high yields of the first crop (wheat) as cow-dung and compost, yet the yields from molasses applied alone or with lime are almost in every case better than the yields from no-manure and in certain cases the difference is statistically significant.

TABLE VI

Effect of molasses and other manures on the yield of wheat crop and their residual effect on the following sanai *crop during the years 1933-34 and 1934-35*

Station and year	Name and quantity of manure applied per acre	Date of application	Date of sowing	Average yield of wheat crop per plot in lbs.		Average yield of subsequent *sanai* crop per plot in lbs.	
				Grain	*Bhusa*	Grain	*Bhusa*
Government Research Farm, Cawnpore, 1933-34	Molasses 2·5 tons per acre	1-7-33	6-11-33	26·7	54·9	12·34	37·15
	Molasses 2·5 tons + lime 0·5 ton per acre.	Do.	Do.	25·2	53·0	13·34	40·70
	Molasses 2·5 tons per acre	25-8-33	Do.	23·2	44·5	13·04	42·12
	Molasses 2·5 tons + lime 0·5 ton per acre.	26-8-33	Do.	26·4	57·4	13·18	41·25
	Cow-dung 2·5 tons per acre	1-9-33	Do.	30·9	73·7	12·58	43·68
	No manure	..	Do.	18·7	50·3	9·88	28·55
Government Research Farm, Cawnpore, 1934-35	Molasses 2·5 tons per acre	4-7-34	25-10-34	30·9	64·6	16·72	84·80
	Molasses 2·5 tons + lime 0·5 ton per acre.	Do.	Do.	30·2	54·7	17·32	87·61
	Molasses 2·5 tons per acre	30-8-34	Do.	23·6	50·3	17·35	86·68
	Molasses 2·5 tons + lime 0·5 ton per acre.	Do.	Do.	27·9	48·5	19·52	83·35
	Compost 40 mds. per acre	29-9-34	Do.	34·5	68·3	19·00	84·35
	No manure	..	Do.	21·4	44·2	16·65	77·05
Government Experimental Farm, Gorakhpur, 1934-35	Molasses 160 mds. per acre	10-10-34	23-10-34	68·3	..	..	..
	No manure	..	Do.	62·0	..	..	..

At Gorakhpur, too, molasses-treated replicates have given greater outturn of wheat grain, though statistically insignificant than the untreated ones.

Looking at the average yields of subsequent crops (sunn-hemp) of two years it is seen that the treated plots whether with cow-dung, compost or molasses have all given better outturns than the controls. In the first year out of four molasses treatments three have given greater yields than even cow-dung and in the second year only one than compost. Thus the data of yields of different subsequent crops obtained from the residual effects of molasses, fertilisers and other manures go to show the superiority of molasses in this respect and confirm the view of the Pioneer Mill Co. that the beneficial effects of molasses persists for several crops and can be ascribed to the enhancement of the processes of nitrogen fixation, ammonification and nitrification brought about in the soils by the application of molasses.

EFFECT ON SOILS

At the end of each harvest the soil of each pot of culture experiments and of each plot of planted canes and wheat fields of Cawnpore Research Farm was examined for total nitrogen according to Kjeldahl's method modified by Gunning and the data obtained are given in Tables VII, VIII and IX.

The two wheat fields of the Cawnpore Research Farm were at a high level and consisted of a slightly alkaline heavy clay soil with *kanker* nodules here and there, giving very poor crops in comparison to other fields of the farm. The application of molasses seems to have improved their texture and fertility as was observed by the tilth of the soils and the growth of the first and subsequent crops.

The last two columns of Table VII give the average percentage of nitrogen in soils of each pot and of each treatment of the pot-culture experiments, and on comparing the figures obtained from untreated pots with those from treated ones the nitrogen contents of soils treated with molasses show an increase, while that of soils treated with fertilisers a decrease, indicating thereby the beneficial effect of molasses on nitrogen content of soils.

TABLE VII

Showing nitrogen contents of soils of pot-culture experiments

Pot Nos.	Treatment	1932-33		1933-34		1934-35		Total	Average percentage of nitrogen in soil of each plot	Average percentage of nitrogen in soil of each treatment
		Percentage of nitrogen in soil		Percentage of nitrogen in soil		Percentage of nitrogen in soil				
		After *Kharif*	After *Rabi*	After *Kharif*	After *Rabi*	After *Kharif*	After *Rabi*			
1A	No manure . . .	0·0504	0·0532	0·0581	0·0580	0·0550	0·0420	0·3167	0·0528	0·0528
1B	Do. . . .	0·0616	0·0546	0·0448	0·0595	0·0530	0·0434	0·3169	0·0528	
2A	Do. . . .	0·0504	0·0490	0·0524	0·0494	0·0464	0·0378	0·2854	0·0475	0·0516
2B	Do. . . .	0·0684	0·0490	0·0584	0·0554	0·0640	0·0392	0·3344	0·0557	
3A	Molasses 6 tons per acre	0·0644	0·0476	0·0521	0·0549	0·0680	0·0392	0·3262	0·0544	0·0539
3B	Do. . . .	0·0574	0·0504	0·0540	0·0546	0·0504	0·0406	0·3074	0·0512	
3C	Do. . . .	0·0560	0·0476	0·0762	0·0573	0·0580	0·0420	0·3371	0·0562	
4A	$(NH_4)_2SO_4$, K_2SO_4, Na_2HPO_4.	0·0504	0·0462	0·0520	0·0494	0·0520	0·0406	0·2906	0·0484	0·0496
4B	Do. . . .	0·0504	0·0504	0·0518	0·0458	0·0520	0·0378	0·2882	0·0480	
4C	Do. . . .	0·0672	0·0490	0·0547	0·0551	0·0504	0·0392	0·3156	0·0526	
5A	K_2SO_4, Na_2HPO_4 . .	0·0532	0·0434	0·0511	0·0491	0·0530	0·0378	0·2876	0·0479	0·0479
5B	Do. . . .	0·0532	0·0462	0·0512	0·0494	0·0500	0·0378	0·2878	0·0480	
5C	Do. . . .	0·0518	0·0462	0·0507	0·0525	0·0460	0·0392	0·2864	0·0477	
6A	$(NH_4)_2SO_4$ . . .	0·0640	0·0476	0·0538	0·0596	0·0470	0·0448	0·3168	0·0528	0·0506
6B	Do. . . .	0·0530	0·0476	0·0547	0·0571	0·0470	0·0420	0·3014	0·0502	
6C	Do. . . .	0·0490	0·0462	0·0468	0·0561	0·0520	0·0420	0·2921	0·0487	
8A	Molasses 12 tons per acre	0·0770	0·0532	0·0623	0·0700	0·0570	0·0448	0·3643	0·0607	0·0618
8B	Do. . . .	0·0810	0·0602	0·0584	0·0673	0·0576	0·0462	0·3701	0·0617	
8C	Do. . . .	0·0770	0·0574	0·0620	0·0707	0·0600	0·0504	0·3775	0·0629	

1A, 1B, and all C had no crop throughout and were kept fallow while 8A and 8B only *Rabi* crops.

TABLE VIII

Showing percentage of nitrogen in soils treated with molasses and fertilisers after harvesting canes from fields of 1933-34 and 1934-35

Time of application of manures	Name of quality of manure applied	Nitrogen in soil of replica I per cent	Nitrogen in soil of replica II per cent	Nitrogen in soil of replica III per cent	Nitrogen in soil of replica IV per cent	Nitrogen in soil of replica V per cent	Total	Average percentage of nitrogen in soil of each treatment
1933-34, 2 months before sowing canes	Molasses 6 tons per acre . .	0·0489	0·0510	0·0602	0·0491	0·0598	0·2690	0·0538
	Molasses 12 tons per acre . .	0·0567	0·0589	0·0494	0·0469	0·0490	0·2609	0·0522
	Fertilisers equivalent to 6 tons molasses per acre.	0·0487	0·0496	0·0672	0·0526	0·0602	0·2783	0·0557
	Fertilisers equivalent to 12 tons molasses per acre.	0·0515	0·0620	0·0664	0·0515	0·0647	0·2961	0·0592
	No manure	0·0491	0·0524	0·0472	0·0561	0·0511	0·2559	0·0512
1933-34, 2 months after sowing canes	Molasses 6 tons per acre . .	0·0610	0·0596	0·0564	0·0550	0·0493	0·2813	0·0563
	Molasses 12 tons per acre . .	0·0580	0·0566	0·0585	0·0580	0·0613	0·2924	0·0585
	Fertilisers equivalent to 6 tons molasses per acre.	0·0665	0·0521	0·0528	0·0571	0·0571	0·2856	0·0571
	Fertilisers equivalent to 12 tons molasses per acre.	0·0623	0·0524	0·0487	0·0644	0·0610	0·2888	0·0578
	No manure	0·0575	0·0602	0·0533	0·0504	0·0591	0·2805	0·0561
1933-34, 2 months before sowing canes	Molasses 6 tons per acre . .	0·0448	0·0420	0·0546	0·0658	0·0588	0·2660	0·0532
	Molasses 12 tons per acre . .	0·0490	0·0350	0·0504	0·0714	0·0518	0·2576	0·0515
	Fertilisers equivalent to 6 tons molasses per acre.	0·0532	0·0420	0·0490	0·0560	0·0686	0·2688	0·0538
	Fertilisers equivalent to 12 tons molasses per acre.	0·0448	0·0350	0·0448	0·0504	0·0686	0·2436	0·0487
	No manure	0·0504	0·0476	0·0546	0·0542	0·0572	0·2640	0·0528
1934-35, 2 months after sowing canes	Molasses 6 tons per acre . .	0·0490	0·0546	0·0672	0·0578	0·0518	0·2804	0·0561
	Molasses 12 tons per acre . .	0·0532	0·0538	0·0546	0·0574	0·0464	0·2704	0·0541
	Fertilisers equivalent to 6 tons molasses per acre.	0·0434	0·0546	0·0630	0·0560	0·0672	0·2842	0·0568
	Fertilisers equivalent to 12 tons molasses per acre.	0·0350	0·0518	0·0630	0·0574	0·0616	0·2688	0·0538
	No manure	0·0504	0·0420	0·0532	0·0616	0·0438	0·2510	0·0502

TABLE IX

Showing percentage of nitrogen found in soils treated with molasses and other manures of wheat fields 1934-35

Treatment	Replica I Nitrogen in soil (per cent)	Replica II Nitrogen in soil (per cent)	Replica III Nitrogen in soil (per cent)	Replica IV Nitrogen in soil (per cent)	Replica V Nitrogen in soil (per cent)	Total	Average percentage of nitrogen in soil of each treatment	Difference between percentage of nitrogen in soils before sowing and harvesting	Increase of nitrogen (per cent)
				1934-35, before sowing wheat					
A	0·0652	0·0434	0·0364	0·0350	0·0378	0·2178	0·0436		
B	0·0560	0·0464	0·0476	0·0392	0·0462	0·2354	0·0471		
C	0·0490	0·0476	0·0532	0·0364	0·0364	0·2226	0·0445		
D	0·0616	0·0546	0·0476	0·0378	0·0378	0·2394	0·0479		
E	0·0602	0·0462	0·0504	0·0392	0·0504	0·2464	0·0493		
Control	0·0616	0·0462	0·0504	0·0350	0·0434	0·2366	0·0473		
				1934-35, after harvesting wheat					
A	0·0474	0·0476	0·0532	0·0558	0·0420	0·2460	0·0492	0·0056	12·8
B	0·0560	0·0546	0·0434	0·0560	0·0392	0·2492	0·0498	0·0027	5·7
C	0·0602	0·0490	0·0518	0·0490	0·0392	0·2492	0·0498	0·0053	11·9
D	0·0504	0·0574	0·0518	0·0448	0·0574	0·2618	0·0523	0·0044	9·2
E	0·0490	0·0490	0·0476	0·0560	0·0490	0·2506	0·0501	0·0008	1·6
Control	0·0546	0·0490	0·0504	0·0448	0·0476	0·2464	0·0493	0·0020	4·2

NOTE.—A—2·5 tons molasses per acre applied in July.

B—2·5 tons molasses per acre applied in July—0·5 ton lime.

C—2·5 tons molasses per acre applied in August.

D—2·5 tons molasses per acre applied in August—0·5 ton lime.

E—40 maunds compost manure per acre applied in September.

Control—No manure applied.

The column of average percentage of nitrogen in soils of each treatment of Table VIII shows that the soils treated with molasses are generally richer in nitrogen than those having no manure.

Table IX gives the average percentage of nitrogen found in soils of differently treated replicates of wheat of 1934-35 before sowing and after harvesting the crop. The column of the increase of nitrogen percentage shows the least increase of nitrogen in soils of replicates to which compost was added and which gave the highest yield of crop, that might have been responsible for the removal of almost all the nitrogen, inherent and cumulative without letting it accumulate in the soil. The average percentage increase of nitrogen, in soils treated with molasses either alone or with lime is always greater than that in soils of control replicates. All the molasses-treated replicates have, on the average, given better outturn of crops and greater increase in nitrogen than the controls, showing thereby that the addition of molasses as manure to soils enhances the outturn of crops and at the same time enriches the soil with nitrogen.

Seeing the effect of molasses on the poor soils of wheat fields another pot-culture experiment was started in July 1934 to see the effect of molasses on different kinds of sterile soils of the Provinces. Three varieties of altogether infertile soils, clayey, calcareous and alkali, were procured and treated with molasses at the rate of six, twelve and eighteen tons per acre. The molasses was dissolved in water, sufficient to bring the moisture content of soils up to twenty per cent and then, mixed with the weighed quantities of different soils, which were filled into duplicate pots, one pot containing the original soil of each variety with twenty per cent moisture being kept as a control. Before sowing the *Rabi* crop the soil of each pot was stirred out every month, weighed and any loss of water was made up. At the end of October nine seeds of wheat were sown in each pot. The number of seeds germinating in each pot and the growth of plants were observed and every week the amount of moisture lost was made up.

There was no germination in untreated soils of each kind as well as in the alkali soil having six tons molasses. In pots of calcareous and clayey soils with six tons molasses per acre about eleven per cent germination took place. In alkali and calcareous soils with twelve and eighteen tons molasses about thirty per cent, while in clayey soil with the same quantities of molasses about fifty per cent seeds germinated. Out of all these germinated plants only those in the calcareous and the clayey soil with eighteen tons molasses developed, tillered and brought forth ears, others died within four months of their germination. The plants of the clayey soil had a better growth than those of calcareous. Thus the application of molasses to these altogether sterile soils seems to have brought about in them a change for the better and indicates the possibility of utilising molasses not only for reclaiming alkali soils as shown by Dhar [1935] but also other varieties of sterile soils.

Table X contains the average nitrogen and sodium carbonate contents and pH values of these treated and untreated soils determined after harvesting wheat. It shows that the soils treated with molasses have not only become richer in nitrogen but also poorer in alkalinity and the more the molasses is applied the greater is the increase in nitrogen and a corresponding decrease in the pH values and the sodium carbonate contents. Thus it appears that the application of molasses can improve the fertility of sterile soils of clayey, calcareous and alkaline characters by increasing their nitrogen contents and at the same time lowering their pH values from alkalinity to neutrality.

TABLE X

Showing nitrogen and sodium carbonate contents and p*H values of treated and untreated sterile soils of pot-culture experiments*

Description of soils	Untreated soils			Soils treated with 6 tons molasses per acre			Soil treated with 12 tons molasses per acre			Soil treated with 18 tons molasses per acre		
	Nitrogen (per cent)	Na_2CO_3 (per cent)	*p*H value	Nitrogen (er c ent)	Na_2CO_3 (per cent)	*p*H value	Nitrogen (per cent)	Na_2CO_3 (per cent)	*p*H value	Nitrogen (per cent)	Na_2CO_3 (per cent)	*p*H value
Clayey soil .	0·0350	0·0053	8·4	0·0420	0·0042	8·2	0·0574	trace	8·1	0·0586	*Nil*	8·1
Calcareous soil .	0·0560	0·0360	8·2	0·0568	0·0223	7·5	0·0770	0·019	7·3	0·0798	0·0042	7·2
Alkali soil . .	0·0060	0·0297	7·7	0·0420	0·0276	7·4	0·0572	0·015	7·3	0·0590	*Nil*	7·2

From the results of experiments described the following conclusions can be drawn :—

1. From the manurial point of view molasses are poor in nitrogen but rich in potash and their ash content varies between five and twelve per cent.
2. The results of pot-culture and field-scale experiments show that crop yields obtained from molasses as manure, particularly when applied at the rate of twelve tons per acre and two months before sowing, compare favourably with those obtained from fertilisers.
3. The outturn of planted canes and sucrose per acre increased with the increase in the quantity of molasses applied two months before planting and at sowing time, while in the case of molasses applied at tillering, the yield of both cane and sucrose decreased. The increased yields of cane obtained from molasses applied two months before sowing at the rate of 180 and 270 maunds per acre over those from controls are statistically significant.
4. The juices of planted canes treated with molasses two months before sowing are not found poorer in sucrose than those of untreated canes or treated with fertilisers.
5. The juices of ratoon canes treated with molasses are generally poorer in sucrose than those of untreated ones, but the tonnage of the former is significantly higher than that of the latter, which offsets the poor quality of the juice.
6. Molasses does not produce as high a yield of wheat crop as does cow-dung or compost, but it gives appreciable increase in the yield in comparison to no-manure.
7. The outturns of different subsequent crops obtained from the residual effects of molasses, fertilisers and other manures show the superiority of molasses over others in this respect.
8. After harvest the soils treated with molasses are generally found richer in nitrogen than those treated with other manures, fertilisers or no-manure, showing thereby that nitrogen fixation is enhanced in soils by the application of molasses.
9. Application of molasses can improve the fertility of sterile soils of clayey, calcareous and alkaline characters by increasing their nitrogen contents and lowering their *p*H values from alkalinity to neutrality.
10. Beneficial results are obtained only when molasses is applied to the soil sometime before sowing the crop and well mixed with it by waterings and hoeings and keeping the soil well aerated.
11. The effect of molasses as manure is more appreciable on poor than on rich soils.

ACKNOWLEDGMENTS

The authors take the opportunity of expressing their thanks to Mr. G. R. Saksena for help particularly in the determination of *p*H values.

REFERENCES

Batham, H. N. and Nigam, L. S. (1930). *Soil Sci.* **29,** 181-190.

Berthelot, M. (1885). *Comptes Rendus* **101,** 775-784.

Cross, W. E. (1911). *I. S. J.* **13,** 191.

Dhar, N. R. and others (1934-35). *Proc. Academy of Sciences, U. P., India,* **4,** 175-178 and 330-341.

Dhar, N. R. (1935). Presidential Address, Academy of Sciences, U. P., India.

Heck, A. F. (1931). *I. S. J.* **33,** 325.

Kuyper, J. (1926). *Archief* **34,** No. 4, 95-98. (*I. S. J.* **28,** 227).

Melaselumestig, G. Booberg (1932). *Archief Deel* **3,** 4, 103. (*I. S. J.* **34,** 56).

Peck, S. S. (1912). *I. S. J.* **14,** 353.

Verret, J. A. and Doty, R. E. (1934). *I. S. J.* **36,** 56.

Viswa Nath, B. (1934). *Report of the Govt. Agric. Chemist, Madras.*

STUDIES ON *SCHISTOCERCA GREGARIA* FORSK. *

IX. SOME OBSERVATIONS ON THE HISTOLOGY OF THE BLOOD OF THE DESERT LOCUST

BY

C. B. MATHUR, M.Sc. (Luck.)

AND

B. N. SONI, B.Sc. (Edin.)

Locust Research Laboratory, Punjab Agricultural College, Lyallpur

(Received for publication on 27th August 1936)

(With Plates XVIII and XIX)

CONTENTS

	Page
I. Introduction	318
II. Method	318
III. Fat globules in the plasma	319
IV. Haematocytes	319
(*a*) Historical	319
(*b*) In *Schistocerca gregaria*	320

---

* Studies on *Schistocerca gregaria* Forsk :—

I.—The micropyle in *Schistocerca gregaria* Forsk. and some other Acridiidæ. *Ind. Jour. Agric. Sci.* **3**, 639-645, 1933.

II.—The biology of the Desert Locust with special relation to temperature. *Ind. Jour. Agric. Sci.* **6**, 188-262, 1936.

III.—Why locusts eat wool. A study in the hydromania of *Schistocerca gregaria*. *Ind. Jour. Agric. Sci.* **6**, 263-267, 1936.

IV.—Colour changes and sexual behaviour in desexualized *Schistocerca gregaria* adults. *Ind. Jour. Agric. Sci.* **6**, 586-590, 1936.

V.—Pigmentation and physical exertion. *Ind. Jour. Agric. Sci.* **6**, 591-623, 1936.

VI.—Influence of temperature on the intensity and extent of black pattern in the Desert Locust hoppers bred crowded. *Ind. Jour. Agric. Sci.* **6**, 624-664, 1936.

VII.—Factors determining the movement of the vermiform larvæ. *Ind. Jour. Agric. Sci.* **6**, 665-671, 1936.

VIII.—Influence of carbon dioxide on development of black pigmentation in *Schistocerca gregaria* Forsk. *Ind. Jour. Agric. Sci.* **6**, 1005-1030, 1936

PAGE
V. MULTIPLICATION OF HAEMATOCYTES 321
VI. NUMBER OF HAEMATOCYTES 322
VII. PHAGOCYTES 322
VIII. CYTOPLASMIC INCLUSIONS 323
(a) Golgi bodies 323
(b) Mitochondria 323
(c) Albuminous granules 323
IX. AMOEBOID MOVEMENTS AND PLASMOLYSIS 324
X. ACKNOWLEDGMENTS 324
XI. SUMMARY 324
REFERENCES 325

INTRODUCTION

THIS paper deals with the hæmatocytes and formed bodies of the body fluid or blood of *Schistocerca gregaria*. The blood of the *solitaria* as well as the *gregaria* locust was studied and it has been determined that so far as these elements are concerned the blood of this locust in the two phases does not appreciably differ. Thus although the following description is based solely on observation on the blood of hoppers and adults of the *gregaria* phase, it applies equally to that of the *solitaria* phase. It might be mentioned that the colour of the plasma and possibly hydrogen-ion concentration differs with the phase.

II. METHOD

The blood can be obtained by pricking any part of the body of the insect. The cervix was found to be as convenient for this purpose as any other region and during the course of this investigation blood was always taken from this region. Blood-films were made for permanent preparations. For study *in vivo* the hanging-drop method was employed. It must be mentioned that after a short exposure the leucocytes begin to undergo distortion, showing all sorts of changes in shape, which are misleading.

Shull and others [1932 ; 1933] in their recent researches on toxic chemicals for insects have shown that certain chemicals when used for killing an insect, not only stop its blood from coagulating for a long time after the insect is dead, but also fix the leucocytes while inside the body. One of these chemicals, namely acetic acid, gave us excellent results. The hæmatocytes of the blood of hoppers killed in acetic acid vapour, retained their natural shape and size and compared very favourably with those seen in fresh hanging-drop preparation of the blood from a living hopper. The size of the hæmatocytes was measured from films prepared from the blood of hoppers so treated. The counting was done by hæmocytometer with Neubauer ruling, after diluting the blood with Turks' fluid, which also served to fix and stain the hæmatocytes.

For fixing blood films, satisfactory results were obtained by the use of osmic acid fumes, Flemming's without acetic acid, Bouin's fluid, corrosive sublimate, and absolute alcohol with ether. For the study of cell inclusions, Da Fano, Gatenby and Altmann methods gave best results.

Among the stains used the most suitable were : Delafield's hæmatoxylin, Heidenhein's iron hæmatoxylin, Leishman and Altmann. Janus green B used as a vital stain showed mitochondria admirably well.

III. FAT GLOBULES IN THE PLASMA

There is often found, suspended in the plasma, a number of globular bodies, which stain brick-red with Sudan III and black with osmic acid. They are thus fatty in nature. The chief point of interest about them is that they are most numerous in the blood of a first-stage hopper. In this stage only a few hæmatocytes are present in the blood and the plasma abounds in fatty bodies. An instar-to-instar study of the hoppers has shown that the number of these fatty globules decreases perceptibly as the hoppers advance in age, so much so that only a few globules are seen in the blood of an advanced fifth-instar hopper and it is in very rare cases indeed that these globules persist in the winged stage. This tends to show that the fat globules, being of a nutritive nature, are almost completely used up, during the hopper stages, in the growth and development of the locust. Bishop [1923] also observes that the blood of an insect may be looked upon as a nutrient medium for the growth of the body tissues during the earlier stages of its life when the globules predominate and serve as nutritive bodies.

IV. THE HAEMATOCYTES

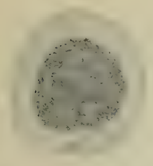
(*a*) *Historical.*—The hæmatocytes are colourless, nucleated cells floating in a transparent fluid, known as plasma. Widely different opinions have been held as to their form and shape. Graber [1891] described leucocytes of six forms in a grasshopper. This view is, however, of historical importance only, since subsequent workers have shown that several of his forms were artifacts. The hæmatocytes possess a very delicate and elastic cell membrane and change their shape under pressure (such as the pressure of the cover glass) or when exposed to the atmosphere or placed in a medium of higher or lower osmotic pressure than the plasma. Wigglesworth [1934] observed that " the extraordinary diversity of appearance was due to the protean forms which the hæmatocytes could assume rather than to the abundance of distinct types ". Cuénot [1895] described four forms for the leucocytes in *Gryllus campestris* and regarded them as four successive stages which a leucocyte passed through during its life period.

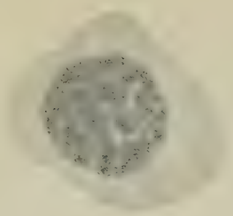
In the first stage the leucocytes were characterised by their very small size. These possessed a very deeply staining nucleus, which occupied almost the whole of the cell. It was at this stage that the leucocytes multiplied. During the second stage the protoplasm of the leucocytes was filled with acidophilous granules, in the third stage they were devoid of these granules and possessed an intense phagocytic power and in the fourth or the final stage they contained acidophilous granules and displayed a marked fatty degeneration.

Later his observations were confirmed by Kollmann [1908]. Hollande [1911] also studied the blood of *Gryllus campestris*. He agreed in general with Cuénot and Kollmann, but he recognised only three forms representing different stages. The first stage of Cuénot was named as ‘ pro-leucocyte ’. It was very frequently seen undergoing mitotic divisions. The second stage of the acidophilous granules of Cuénot was called granular leucocyte and the third stage, having no granules but possessing an extensive phagocytic power, was called phagocyte. He did not recognise the fourth stage of Cuénot, characterised by fatty degeneration, and stated that the granular leucocytes as well as the phagocytes could undergo fatty degeneration without ever entering the phagocyte stage. Muttkowski [1924] recognised two types of leucocytes in the blood of an insect : amoebocytes (large lightly staining leucocyte cells) and chromophils (smaller, darker), arising from a common primary cell element. Haber [1926] agreed with this view and pointed out that the vacuolated large amoebocytes were degenerating cells. Bogojawlensky [1932] named the primary cell elements which gave rise to the two types of cells as the ‘ mother cells ’. Pilat [1935] recognised haematocytes of several stages in the life of a blood cell. He stated " The normal picture of the blood of the locust presents different stages in the development of the haemocytes, beginning with youngest forms (cells of 8-9 μ) and ending with decaying and perishing forms represented by cells with red nuclei."

(*b*) *In Schistocerca gregaria.*—The haematocytes in the Desert Locust from the point of view of shape can be placed in two categories, *viz.* (*a*) round cells and (*b*) pyriform leucocytes. The former are undifferentiated spherical cells and are characterised by their small size. Bogojawlensky [1932] has appropriately designated such cells as the ‘ mother cells ’. It is only at this stage that the haematocytes undergo a division. The mother cell (Plate XVIII, figs. 1 and 2) measures 8-12 μ. Its nucleus stains deeply and occupies practically the whole of the cell there being only a thin layer of cytoplasm around it. As a rule the nucleus has a single well-defined nucleolus. Sometimes, however, larger round cells are also met with but these are the ones that are undergoing fatty degeneration. Their cytoplasm has a vacuolated appearance and the nucleus takes a dull stain with haematoxylin (Plate XVIII, fig. 3).

The pyriform haematocytes may be looked upon as the true leucocytes. These are spindle shaped, but compressed from side to side (Plate XVIII, figs. 4, 5). Likewise, their nuclei are compressed. Thus the cytoplasmic space left between the nucleus and the cell membrane is about the same all round, the nucleus being centrally placed.





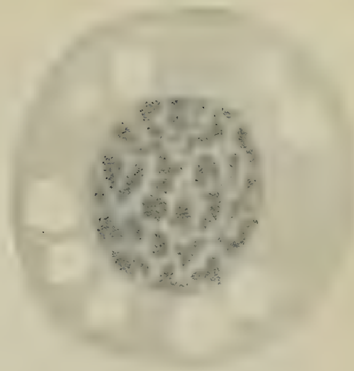


Fig. 3

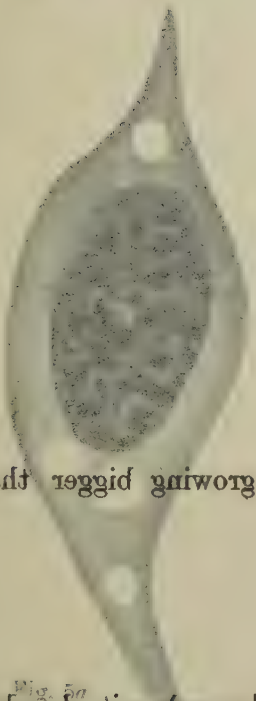


Fig. 5a

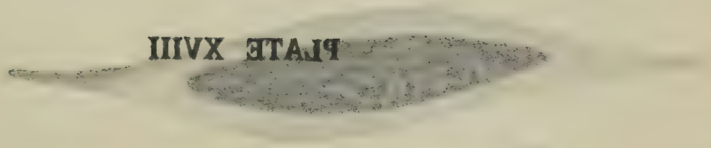
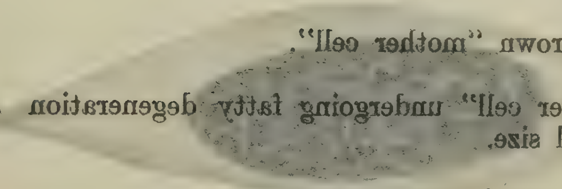


Fig. 4



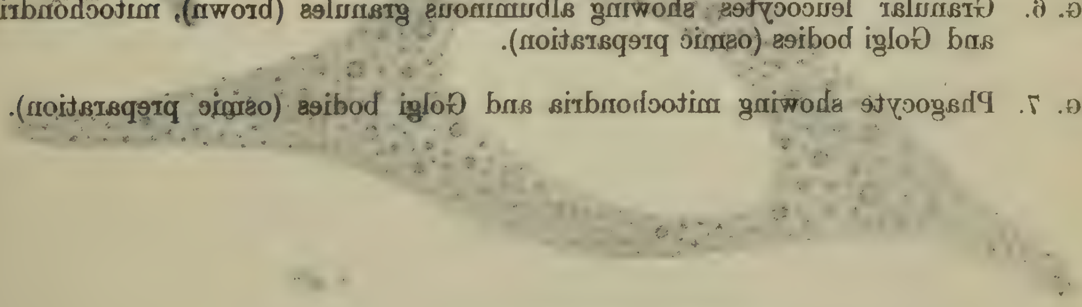


PLATE XVIII

FIG. 1. Young "mother cell".

FIG. 2. Full grown "mother cell".

FIG. 3. "Mother cell" undergoing fatty degeneration after growing bigger than normal size.

FIGS. 4 and 5. Pro-leucocyte, two views.

FIG. *5a*. Pro-leucocyte undergoing fatty degeneration.

FIG. 6. Granular leucocytes showing albuminous granules (brown), mitochondria and Golgi bodies (osmic preparation).

FIG. 7. Phagocyte showing mitochondria and Golgi bodies (osmic preparation).

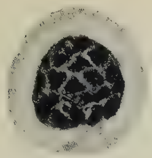


Fig. 1

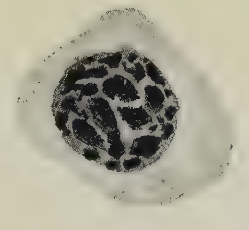


Fig. 2

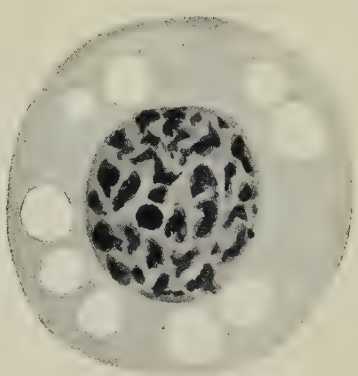


Fig. 3

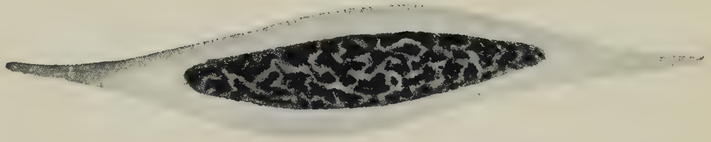


Fig. 4

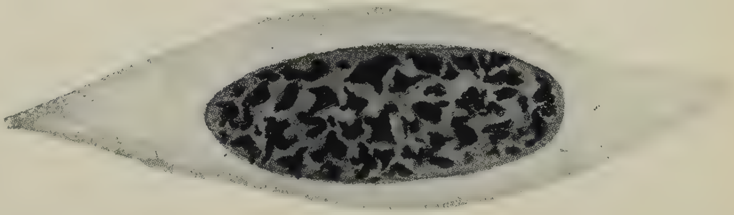


Fig. 5

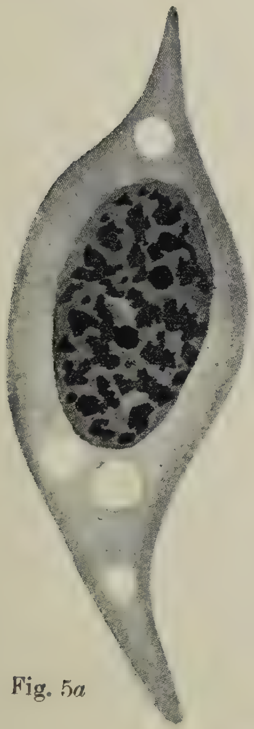


Fig. 5a

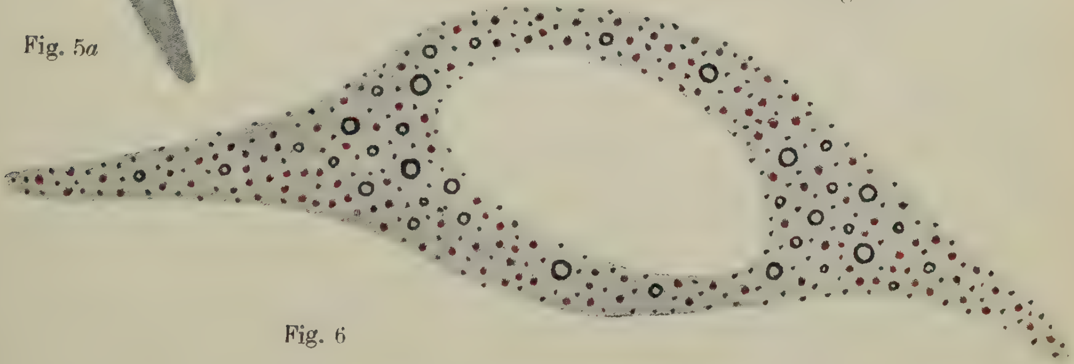
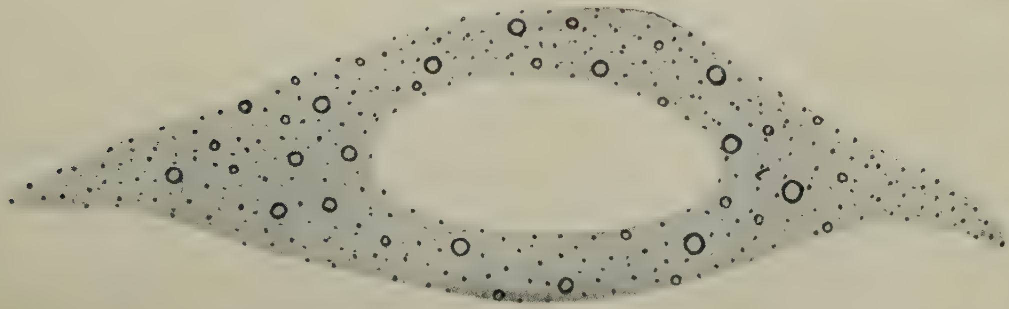


Fig. 6



The pyriform leucocytes can be distinguished into three well-defined types : (*i*) pro-leucocytes (Plate XVIII, figs. 4, 5) (*ii*) the granular leucocytes (Plate XVIII fig. 6) and (*iii*) the phagocytes (Plate XVIII, fig. 7). They differ both in certain morphological characters and in function. The pro-leucocytes arise from the 'mother cells' and give rise to the granular leucocyte as well as the phagocytes. It is a differentiated cell so far as its shape is concerned, but in function it is only a transitory form lying between the 'mother cell' on the one hand and the granular leucocytes and the phagocytes on the other. It is 10-18 μ lorg and in breadth 2-3 μ along its short axis and 5-6 μ along the long axis. It shows no definite character in earlier stages, by which one could foretell the type into which the pro-leucocyte is going to develop. When a pro-leucocyte has grown to about its full size (about 18 μ long) it begins to assume the characters of the next stage, namely the mitochondria of the pro-leucocyte that is to change into a granular leucocyte begin to swell up and give the cytoplasm a granulated appearance. The proleucocytes, in which the mitochondria maintain their original size, develop into phagocytes. Thus the granular leucocytes and the phagocytes are the two types of leucocytes both of which arise from the pro-leucocyte.

In point of size and shape the granular leucocytes and the phagocytes are almost alike. Their maximum size is about 40 μ long and about 16 μ broad. During all the stages of the development of a locust, leucocytes (granular leucocytes and phagocytes) of intermediate sizes are found in abundance, showing that new leucocytes are being formed throughout the life of the Desert Locust.

V. MULTIPLICATION OF HAEMATOCYTES

It is only the mother cells that divide. This division is always mitotic and takes place in all the stages during the development of the locust. The mother cell undergoes division after having attained its full size. Some of the important and commoner phases of this division are shown in Plate XIX, fig. 8 *a-g*.

Cell division is more common in the earlier hopper stages and is governed by several factors, the more important ones being (*a*) age of instar, (*b*) temperature and (*c*) nutrition. The largest number of dividing cells was observed in hoppers breeding at 35° C., while starvation and low temperature appeared to inhibit cell division.

A mother cell after dividing may continue the rôle of a mother cell or it may change into a pro-leucocyte. This tendency becomes evident in the late telophase. When the daughter cells are to play the rôle of a 'mother cell', their outline at the bridge becomes rounded soon after the division is completed. (Plate XIX, fig. 8 *f*), but if they are to form the pro-leucocytes, they form one of the poles of the spindle-shaped pro-leucocytes. The other pole of each daughter cell also begins to take this shape at this juncture (Plate XIX, fig. 8 *g*).

There is no amitotic division. Sometimes, however, there occur a few leucocytes in which the nucleus is lobed (Plate XIX, fig. 9), giving the impression of amitotic division. A detailed study has shown that such cells do not undergo division and, as Agar [1920] has observed " such a condition is merely a temporary lobing or fragmentation of the nucleus without a final division of the cell ". It would appear that the cases of amitotic division mentioned by Cuénot and Hollande refer to such a condition of a nucleus.

VI. NUMBER OF HAEMATOCYTES

The study of the number of haematocytes per unit volume of blood in the various stages of the Desert Locust has given some interesting figures. In the following table are given five observations for each instar of the locust. It shows that the number of haematocytes for a certain volume of blood is characteristic of each instar and that this number rises with each stage. From about 200 haematocytes per cubic millimeter in a first stage hopper the number of leucocytes increases to over 6,000 in the adult.

Number of haematocytes per cubic mm. in the blood of the Desert Locust

Serial No.	Stages					
	I	II	III	IV	V	Adult(early)
1	200	350	1,150	3,500	4,500	6,500
2	200	400	1,200	3,000	5,000	6,000
3	200	400	1,100	3,000	5,000	6,000
4	250	400	1,200	3,500	5,500	6,500
5	250	400	1,250	3,000	5,000	6,500
Average .	220	390	1,180	3,000	5,000	6,300

VII. PHAGOCYTES

The phagocytes and the granular leucocytes are so much alike in form and size that it is not ordinarily possible to differentiate between the two in the living condition under the microscope. However, when the light is partially cut off, the granules in the cytoplasm of the ' granular leucocytes ' become visible and by the negative character, *viz.*, the absence of these granular bodies, the phagocytes can be differentiated. Their phagocytic nature was experimentally tested and it was verified that the granular leucocytes possessed no phagocytic properties. In a fifth stage hopper a 1 : 1,000 mixture of carmine in normal physiological solution was injected by means of a fine hypodermic syringe. After an interval of about two hours a drop of blood was obtained from the treated hopper, fixed in alcohol ether or F. W. A. and lightly stained with iron-haematoxylin. Some of the leucocytes (and they were all non-granular) showed carmine particles engulfed in

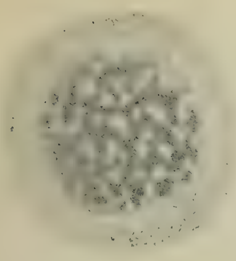


Fig. 8c

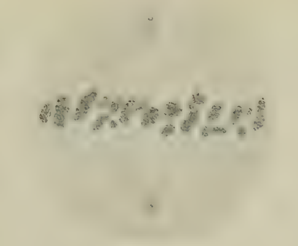
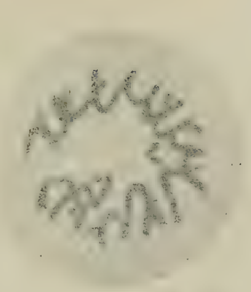


Fig. 8d



[illegible]

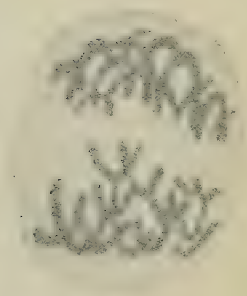


Fig. [illegible]

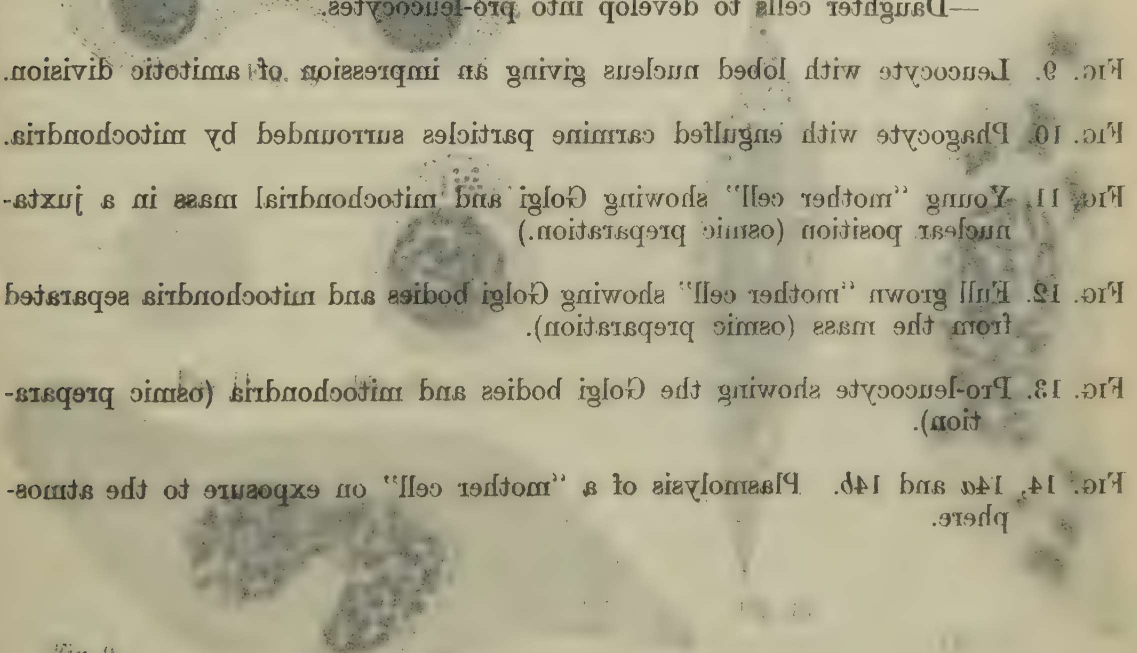


Fig. 9

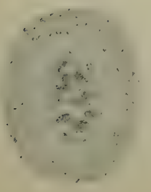


Fig. 14a

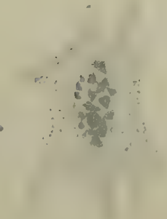


Fig. 14b

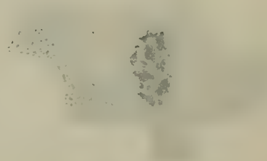


Fig. 14c

PLATE XIX

FIG. 8. Some phases of mitotic division of 'mother cell'. (*a*) 'Mother cell' ready for mitotic division. (*b*) Late prophase. (*c*) Early anaphase (dorsal view). (*d*) Early anaphase (lateral view). (*e*) Late anaphase. (*f*) Telophase—Daughter cells to develop into "mother cells". (*g*) Telophase—Daughter cells to develop into pro-leucocytes.

FIG. 9. Leucocyte with lobed nucleus giving an impression of amitotic division.

FIG. 10. Phagocyte with engulfed carmine particles surrounded by mitochondria.

FIG. 11. Young "mother cell" showing Golgi and mitochondrial mass in a juxta-nuclear position (osmic preparation).

FIG. 12. Full grown "mother cell" showing Golgi bodies and mitochondria separated from the mass (osmic preparation).

FIG. 13. Pro-leucocyte showing the Golgi bodies and mitochondria (osmic preparation).

FIG. 14, 14*a* and 14*b*. Plasmolysis of a "mother cell" on exposure to the atmosphere.

PLATE XIX

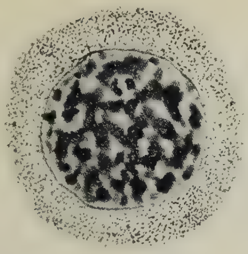


Fig. 8*a*

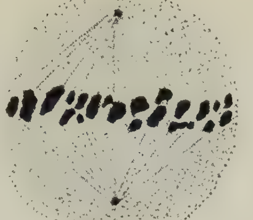


Fig. 8*b*

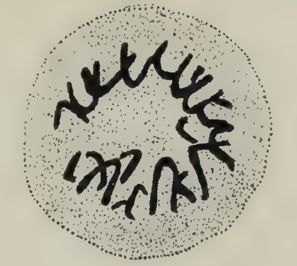


Fig. 8*c*

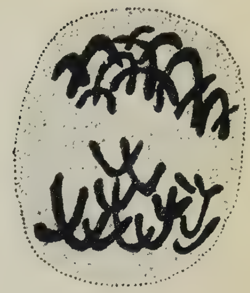


Fig. 8*d*

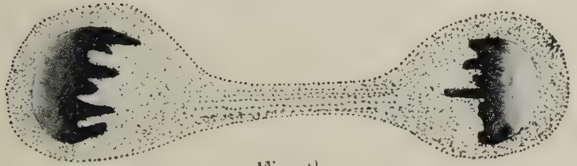


Fig. 8*e*

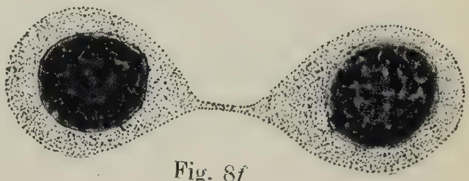


Fig. 8*f*

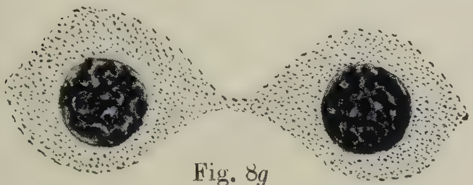


Fig. 8*g*

Fig. 11

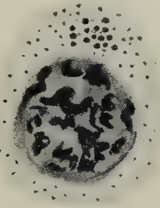


Fig. 12

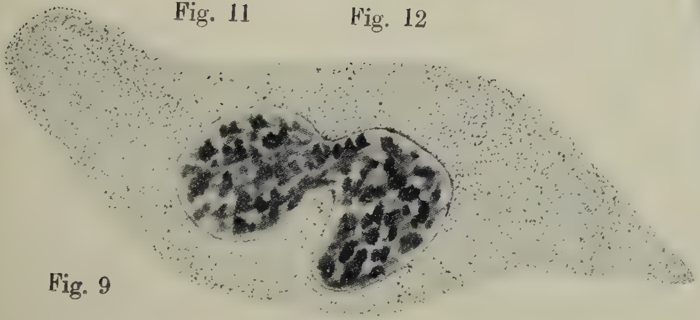


Fig. 9

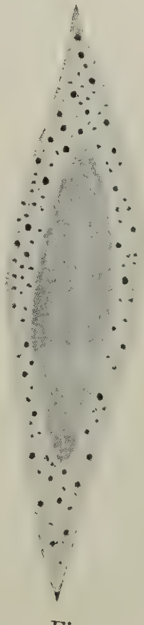


Fig. 13

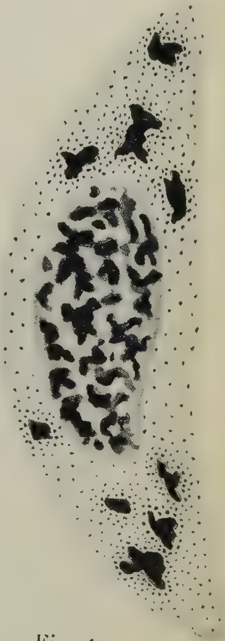


Fig. 10

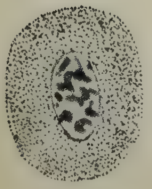


Fig. 14*a*

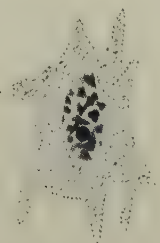


Fig. 14*b*

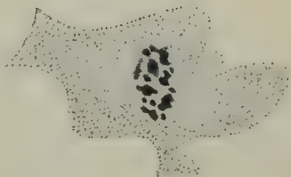


Fig. 14*c*

their cytoplasm (Plate XIX, fig. 10). Of the phagocytes some contained as many as twelve of these particles. The phagocytes constitute about ten per cent of the total number of haematocytes in a fifth stage hopper.

VIII. CYTOPLASMIC INCLUSIONS

(*a*) *Golgi bodies*.—Golgi bodies are present in all haematocytes. In a young mother cell the Golgi bodies are found concentrated in a small juxta-nuclear mass (Plate XIX, fig. 11) and at this stage its elements are not differentiated. As the cell grows the elements separate and establish their identities by the time the cell is ready to divide. They do not disperse throughout the cell but remain restricted to the original site (Plate XIX, fig. 12).

In the pro-leucocytes the Golgi bodies are found uniformly scattered throughout the cytoplasm. In osmic as well as silver preparations they appear as small deep black spherules (Plate XIX, fig. 13).

The Golgi bodies of granular leucocytes (Plate XVIII, fig. 6) and the phagocytes (Plate XVIII, fig. 7) are bigger in size. Here also they are irregularly distributed throughout the cytoplasm. Their size is variable. In a Da Fano preparation the smaller ones appear dot-like while the bigger ones appear as ring-like structures, the rim being deep black and the interior almost colourless. It appears that these bodies are vesicular in structure.

(*b*) *Mitochondria*.—The mitochondria also are present in all haematocytes and appear to play an important part in their physiology. In the younger mother cells (Plate XIX, fig. 11) they are concentrated around the mass of Golgi bodies and together with these give the appearance of what is called the 'yolk nucleus of Balbiani' in the young egg cells of most lower animals. In advanced mother cells (Plate XIX, fig. 12) and in subsequent leucocyte stages they are scattered throughout the cytoplasm.

The cytoplasm of the phagocytes (Plate XVIII, fig. 7) abounds in mitochondria, which are easily dissolved in fixatives containing acetic acid, thus giving the cytoplasm a non-granular appearance. The mitochondria appear to help the cell in its phagocytic action. Foreign particles, such as carmine particles taken in by phagocytes, are seen to be surrounded each by many of the mitochondria which form a dense ring around them (Plate XIX, fig. 10). The number of mitochondria in a granular leucocyte as compared with those in a phagocyte is relatively very small. They lie in the interspaces of the comparatively bigger albuminous granules described below.

(*c*) *Albuminous granules*.—These granules are present in the granular leucocytes only and are really nothing but very much hypertrophied mitochondria. In all granular leucocytes, though more particularly in less advanced stages, one can observe granules of all grades from normal mitochondria to the full-sized albuminous granules. This shows that these granules arise by the growth and accumulation of proteid matter in mitochondria. It would thus appear that the granules serve as nutrition storage bodies. It might be added that these granules, unlike mitochondria, are perfectly fixed by almost any fixative and being more refractile than the cytoplasm can be seen in a living cell by means of dark-field illumination even without the aid of vital stains.

IX. Amoeboid movements and plasmolysis

The haematocytes at no stage possess amoeboid movements, but sometimes there appear cells with protoplasmic projections giving the impression of pseudopodia. These are, however, artifacts and generally arise as follows :—

When a hanging drop of blood has been exposed to air for some time, the haematocytes begin to undergo plasmolysis. In the earlier stage of this process the haematocytes, chiefly the mother cells, begin to change their shape on account of the irregular flow of the cytoplasm. The cytoplasm at first flows in one direction and then in another giving a picture very much akin to amoeboid movement. After a little while these pseudopodia-like projections can no longer be completely withdrawn and as the cytoplasm flows in another direction, new pseudopodia-like projections appear, so that a number of these are seen at a time. Such movements give the incorrect impression of leucocytes showing amoeboid movements. The plasmolysis is complete when one or more of these protoplasmic projections rupture and the cytoplasm flows out (Plate XIX, fig. 14 *a*, *b*, *c*).

X. Acknowledgments

This work was taken up at the suggestion and under the supervision of Khan Bahadur M. Afzal Husain, M.A., I.A.S., then Locust Research Entomologist to the Imperial Council of Agricultural Research. We take this opportunity of tendering our sincere thanks to him for the help given to us. The Imperial Council of Agricultural Research granted to the junior author an honorarium for doing this work. The generosity of the Imperial Council of Agricultural Research is gratefully acknowledged.

XI. Summary

The haematocytes in the Desert Locust are of four distinct types :—

(*a*) 'Mother cells', (*b*) pro-leucocytes, (*c*) granular leucocytes and (*d*) phagocytes. The mother cells are round, while the remaining three forms are pyriform.

The geneology of the haematocytes is graphically represented as follows :—

$$\text{Mother cell} \rightarrow \text{Mother cell} \rightarrow \text{Pro-leucocyte} < \begin{matrix}\text{Granular leucocytes} \\ \text{Phagocytes}\end{matrix}$$

The multiplication of the haematocytes always takes place by mitotic division of the mother cells alone.

The mitochondria in the phagocytes help in their phagocytic activities.

In the granular leucocytes the granules are hypertrophied mitochondria, and serve as nutrition storage bodies.

Each hopper stage has a characteristic number of haematocytes for a certain volume of blood.

There is no amitotic division.

The leucocytes are not capable of amoeboid movement.

REFERENCES

Agar, W. F. (1920). ‘ Cytology with special reference to metazoan nucleus ’, pp. 224. London.

Bishop, G. H. (1923). *Jour. Morph.* (*Philadelphia*) **37**, 533-553.

Bogojawlensky, K. S. (1923). *Arch. russes. Anat. Hist. et. Embryol.* **11**.

Cuénot, L. (1895). *Arch. Biol.* **14**, 293-341.

Graber (1891). *Biol. Centrabl.* **11**, 212.

Haber, V. R. (1926). *Bull. Brooklyn Ent. Soc.* **21**, 61-100.

Hollande, A. Ch. (1911). *Arch. Zool. Paris* (*Ser. 5*) **6**, 283-323.

Kollmann, Max. (1908). *Ann. Sci. Nat. Zool.* **14**, 1-240.

Muttkowski, R. A. (1924). *Bull. Brooklyn Ent. Soc.* **19**, 4-19 and 128-144.

Pilat, M. (1935). *Bull. Ent. Res.* **26**, 283-288.

Shull, W. E., Riley, M. K. and Richardson, C. H. (1932). *Jour. Econ. Ent.* (*Geneva, N. Y.*) **25**, 1070-1072.

Shull, W. E. and Rice, P. L. (1933). *Jour. Econ. Ent.* (*Geneva, N. Y.*) **26**, 1083-1089.

Wigglesworth, V. B. (1934). ‘ Insect physiology ’, pp. 134. London.

PLANT-GROWTH IN RELATION TO HYDROGEN-ION CHANGES IN ITS MEDIUM

BY

B. N. SINGH, D.Sc.

AND

G. P. MITRA, M.Sc.

(*Contribution from the Institute of Agricultural Research, Benares Hindu University.*)

(Received for publication on 24th March 1936)

(With Plates XX and XXI and one text-figure)

CONTENTS

	PAGE
I. INTRODUCTION	327
II. EXPERIMENTAL	328
III. DATA AND DISCUSSION	330
1. Optimal *p*H range for plant-growth	330
2. Morphological characteristics under varying *p*H	333
3. H-ion changes in relation to plant-growth	334
IV. SUMMARY AND CONCLUSIONS	346
REFERENCES	346

I. INTRODUCTION

BIOLOGISTS have realised that the functional activities of living matter are in some manner related to hydrogen-ion concentration. In higher animals, the physiological importance of maintaining a limited range of hydrogen-ion concentration in the blood plasma for regulating the growth and development was early recognised by Lundsgaard [1912], Hasselbach [1923] and Hasselbach and Gammeltoft [1923].

Arrhenius [1922] studied the growth of crop plants under media of varying H-ion concentrations and concluded that acidity or alkalinity of the substratum has a distinct influence on the growth response of plants, depending upon the active number of H-ions present. Investigations prior to that of Arrhenius, conducted by Heald [1896], Kahlenberg and True [1896], Hartwell and Pember [1907], Breazeale and Leclerc [1912], Dachnowski [1914], and Miyaki [1914] relating to the growth of plants in a liquid medium containing appropriate quantities of acid or base, irrespective of the presence or absence of nutrients, lack in interest, since little regard has been paid to the concentration of hydrogen-ions in the solution. Only recently Hoagland [1923] and Bryan [1923] studied the growth of *Triticum* seedlings in nutrient solutions of different hydrogen-ion concentrations. Olsen [1923] has shown the significance of a definite *p*H range in the natural distribution of plants. Weis [1919] and Joffe [1920] studied the growth of plants in relation to the *p*H of the nutrient medium and observed alterations in the hydrogen-ion concentration of the culture medium. Stelmach [1926] and Ulehla [1928] have likewise observed the influence of plant cells in changing the reaction of the medium. In a recent investigation Koslowska [1934] found identical variations in the *p*H of the growing substratum and indicated the property of plant cells to be the casual factor in bringing about such modifications.

A review of the literature thus indicates, that although the property of plant cells in changing the H-ion concentration of the medium during growth has been recognised, the relation of the *p*H of the plant sap and that of the culture solution to each other, and their significance on subsequent growth variations have not been properly studied. It has been the object throughout this investigation to throw light on this aspect of the problem and to determine the *p*H range within which maximum growth of plants is obtainable. Recourse is taken to water-culture experiments under carefully controlled laboratory conditions, later extended to plants grown in natural substratum—the soil.

II. Experimental

Pure strains of *Triticum vulgare* (var. Pusa 4 and 12), *Cajanus indicus* (var. E. B 3 Nagpur), *Crotalaria juncea* (var. Local), *Cicer arietinum* (var. Pusa 46), *Ricinus communis* (var. Local), *Phaseolus vulgaris* (var. Red Altapati, White Altapati, and Black Altapati), *Allium sativum* (var. Type, Lassoon Shapheda), *Lycopersicum esculentum* (var. Cooper's special), *Oryza sativa* (var. P. N 1 (1) Dacca, Deep water) and *Hordeum hexastichum* (var. Pusa 21), were selected for experimentation.

Seeds of as uniform size, weight, and relative density as possible were sterilized by 0·1 per cent mercuric chloride and germinated on mosquito nets tied to glass troughs, receiving a continuous flow of tap water, such that it just touched the lower surface of seeds supplying the necessary moisture. To obtain equilibrium the seedlings of same age and average vigour were placed in Knop's nutrient for a minimum period of forty-eight hours when the cotyledonary leaves were well developed and transferred finally to one-litre culture jars of hard resistant glass containing solutions of different H-ion concentrations. In the Knop's solutions, the nutrients were supplied along with acid phosphate of sodium in a dilution of 0·04 grm. per litre in order to attain the concentration possessing the properties of a buffer.

The H-ion concentration of the solutions was varied after the method of Olsen [1923] and Davidson and Shive [1934] by adding hydrochloric acid or potassium hydroxide in requisite quantities. Every experimental crop was grown in thirty sets of cultures, repeated in triplicate, of varying initial concentrations ranging between *p*H 3·0 and 8·5. Each jar was provided with only three seedlings. The entire population was allowed to grow at a constant temperature of 28° C., maintained by keeping the culture jars immersed in a bath regulated at that temperature. An overhead Philips electric lamp of 1,500 watts provided illumination of constant intensity adjusted to such a height that the illumination neither limited the photosynthetic rate, nor affected the temperature of the bath. The culture solution was aerated from day to day by bubbling air with a suction pump and was changed every third day to maintain a uniform supply of nutrients. The H-ion concentration after every renewal of the solution was kept at the same *p*H at which the plants grew initially. The *p*H values of the cultures were estimated six hourly by the electrometric method using quinhydrone and hydrogen electrodes at every renewal of the solution. The final *p*H values were calculated as the average of others obtained every third day. The degree of buffering of the sap of the plants growing in the culture medium was recorded at corresponding periods of growth. To 4 c.c. of the sap whose degree of *p*H was examined by the electrometric method, the required quantity of centinormal hydrochloric acid or potassium hydroxide was added until the acidity had attained the corresponding degree of changed *p*H. The degree of buffering was then calculated on the basis of the following ratio [Koslowska, 1934] :—

$$\text{Degree of buffering} = \frac{\text{Number of grams of acid or base added to one litre of the solution required to cause the ascertained change of } p\text{H}}{\text{Change of degree of } p\text{H}}$$

The length of tops and roots, the fresh weight and other morphological variabilities were noted fortnightly. The material obtained was subsequently incubated to a constant dry weight, in a steam oven regulated at 100° C.

In order to study the change brought about in the *p*H of the soil by plants growing in the field, for each crop half a dozen lysimeters 5 ft. × 5 ft. × 5 ft. with a 6 ft. border on all sides, were dug temporarily at various points on the Experimental Farm. With a view to providing the optimal conditions for plant growth, the lysimeters thus prepared were properly manured and the soil ultimately mixed to give more or less a homogenous substratum. From each lysimeter twenty-five samples were taken at random to represent the distribution, from a depth of eight inches, and the initial *p*H determined individually for a computation of mean and probability. The crops were subsequently raised and at harvesting time twenty-five random samples were again taken from the neighbourhood of the root-systems of plants growing in each lysimeter and their final *p*H estimated. These values too, were statistically treated and the probable error of the difference of means of the 'initial' and 'final' *p*H calculated to have a fair estimate of significance.

III. DATA AND DISCUSSIONS

1. *Optimal* pH *range for plant growth*

In case of *Triticum* maximal and minimal growths measured on the basis of length of root and shoot are located against more or less same H-ion concentration. There is a well-marked correlation between the initial and final *p*H values of the medium. An increase in the concentration of the former is accompanied by a rise in the latter, though there may not exist an exact proportionality in the two cases throughout the wide range of *p*H used. The *p*H requirement range for maximal plant growth of *Triticum vulgare* (Pusa 4) lies between 6·4 and 6·5. In case of the variety Pusa 12, however, growth is observed over a wider range of *p*H in contrast to Pusa 4, the optimum values being located between 5·7 and 6·6 (Table I).

In *Cajanus indicus* the final *p*H values show great fluctuations with more or less similar variations in the growth of root and shoot (Table III). The optimal range of *p*H for *Cajanus* (Table I) seems to lie between 3·4 and 4·4 (*cf.* values for *p*H obtained in soil for these very plants, Table V), showing thereby the acid-loving property of the plant at least under the present conditions.

Crotalaria juncea is found to grow best between *p*H 6·1 and 6·5 (Table I.) In contrast to *Cajanus*, a characteristic decline is noted in the growth behaviour on both sides of the optimal range, indicating the harmful influence beyond this specific limit.

Cicer arietinum has its own peculiarities and exhibits a number of growth humps in solutions of *p*H beyond 4·5 in contrast to a single hump in lower concentrations. It appears, therefore, that for the growth of *Cicer* decreasing *p*H values below that of the normal are more injurious than the increasing ones (Table III). The optimal *p*H range, however, lies between *p*H 4·4 to 4·8 for root and 4·4 to 5·4 for shoot.

Ricinus communis shows wide fluctuations in growth, which is more marked under higher alkalinity or lesser H-ions, the optimum range being *p*H 5·7—6·2 for roots and 4·6—5·9 for shoots. *Ricinus* appears to be less sensitive than either *Cajanus* or *Cicer* to variations in H-ion concentration and is found to grow over a wider range of *p*H, *viz.*, 4·6—6·2 (less acidic medium).

In *Oryza sativa* too, the *p*H varies in accordance with the pitch of the growth and the correlation between the two is more characteristic than in any of the above types(Table III.) Highest growth is recorded from *p*H 7·8—7·9 (Table I). *Oryza*, therefore, endures sufficiently high alkalinity, probably due to its marshy habitat, where alkalies may be in greater quantity. This agrees with the observation of Mitra and Phukan [1929].

In contrast to the above types, *Allium sativum* grows best under two concentrations, one at *p*H 4·8 and another at *p*H 5·1, and on either side of these points a fall is exhibited which is more abrupt than that obtained for *Crotalaria*. The optimal range is wider as compared to *Oryza* and is located between *p*H 4·8 and 5·1 (Table I).

Lycopersicum esculentum exhibits two maxima under initial *p*H 3·8 and 4·95 which are finally changed to 4·3 and 4·9, respectively (Table III).

The optimal range for *Hordeum hexastichum* lies between *p*H 5·3 and 5·8.

Phaseolus vulgaris (var. Altapati White) shows an endurance to longer range of H-ion concentrations with the increasing alkalinity of the medium. The growth maxima are obtained at *p*H 4·3 and 4·4. A declining growth is only noticeable after an initial *p*H 7·5 (Table III). The plants thus, appear to endure greater alkalinity although growth is not optimum at these points. Red Altapati exhibits lesser fluctuations of growth with maxima at *p*H 4·4 and 4·9. This variety appears to grow well in a wider range of *p*H, although it does not endure as high a concentration of alkali as the white variety. Black Altapati on the other hand, provides a regular growth with very few fluctuations, having characteristic maxima at *p*H 4·1 and 5·0—a range wider than that of the other two varieties (Tables I and III).

It is evident, therefore, that different plants differ in their response to H-ion concentrations, exhibiting specific ranges for their maximum growth. The initial *p*H of the culture solutions, after the plants have grown for a certain time, changes considerably in accordance with the nature of growth, the optimal values being indicated by the final *p*H of the solutions at which the plants exhibit maximum growth (Table I).

TABLE I

Optimal pH *range* *

No.	Plants	Range of *p*H for maximum growth
1	*Oryza sativa* [Deep water paddy P. N 1 (1) Dacca] . .	7·8—7·9
2	*Triticum vulgare* (Pusa 4)	6·4—6·5
3	*Crotalaria juncea* (Local)	6·1—6·5
4	*Triticum vulgare* (Pusa 12)	5·7—6·6
5	*Ricinus communis* (Local)	5·7—6·2
6	*Hordeum hexastichum* (Pusa 21)	5·3—5·8
7	*Phaseolus vulgaris* (Red variety)	4·4—4·9
8	*Phaseolus vulgaris* (White variety)	4·3—4·4
9	*Phaseolus vulgaris* (Black variety)	4·1—5·0
10	*Allium sativum* (Lassoon Shapheda)	4·8—5·1
11	*Lycopersicum esculentum pyriforme*	4·3—4·9
12	*Cicer arietinum* (Pusa 46)	4·4—5·4
13	*Cajanus indicus* (E. B. 3 Nagpur)	3·4—4·4

* The *p*H values indicate the average of the three cultures.

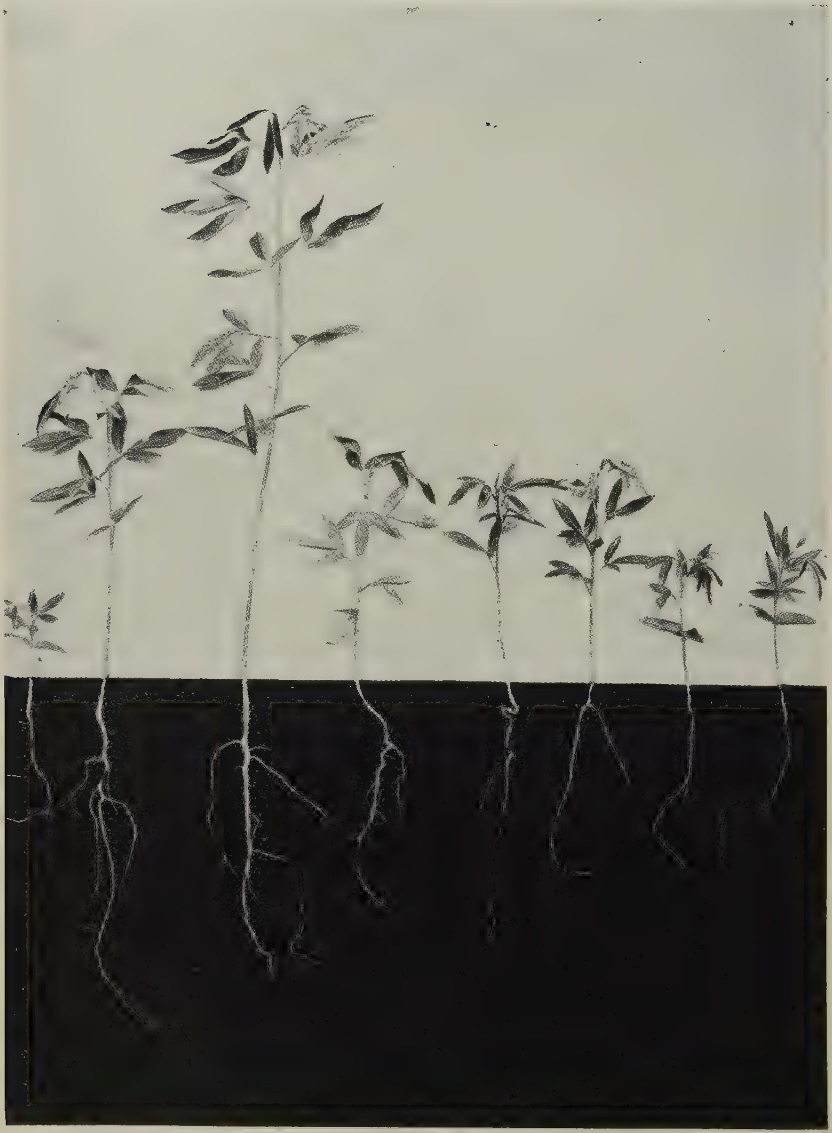
The majority of plants, thus experimented upon, thrive well in acidic medium below *p*H 7. Acid reaction, therefore, seems to be congenial for better development of these plants except *Oryza* which grows well on alkaline medium of *p*H 7·9. A further analysis of these observations indicates that *Phaseolus vulgaris*, *Lycopersicum esculentum*, *Cajanus indicus*, and *Cicer arietinum* are typically acid-loving, growing well within a range of *p*H 3·3—5·4. The rest of the plants (Table I) grow well in a slightly less acidic medium, *Oryza* being the only exception.

Mention may be made in this connection of the work of Olsen [1923] who, while working in Denmark on the natural distribution of plants in relation to their optimal *p*H ranges, found that typical acid-loving plants have optimum growth ranges between *p*H 4·5 and 5·0. Hoagland [1919, 1926] also indicated that slight acidity (*p*H 5 to 7) is not in itself injurious to various types of plants, but alkaline reactions above *p*H 8 are definitely harmful to most of the crop plants.

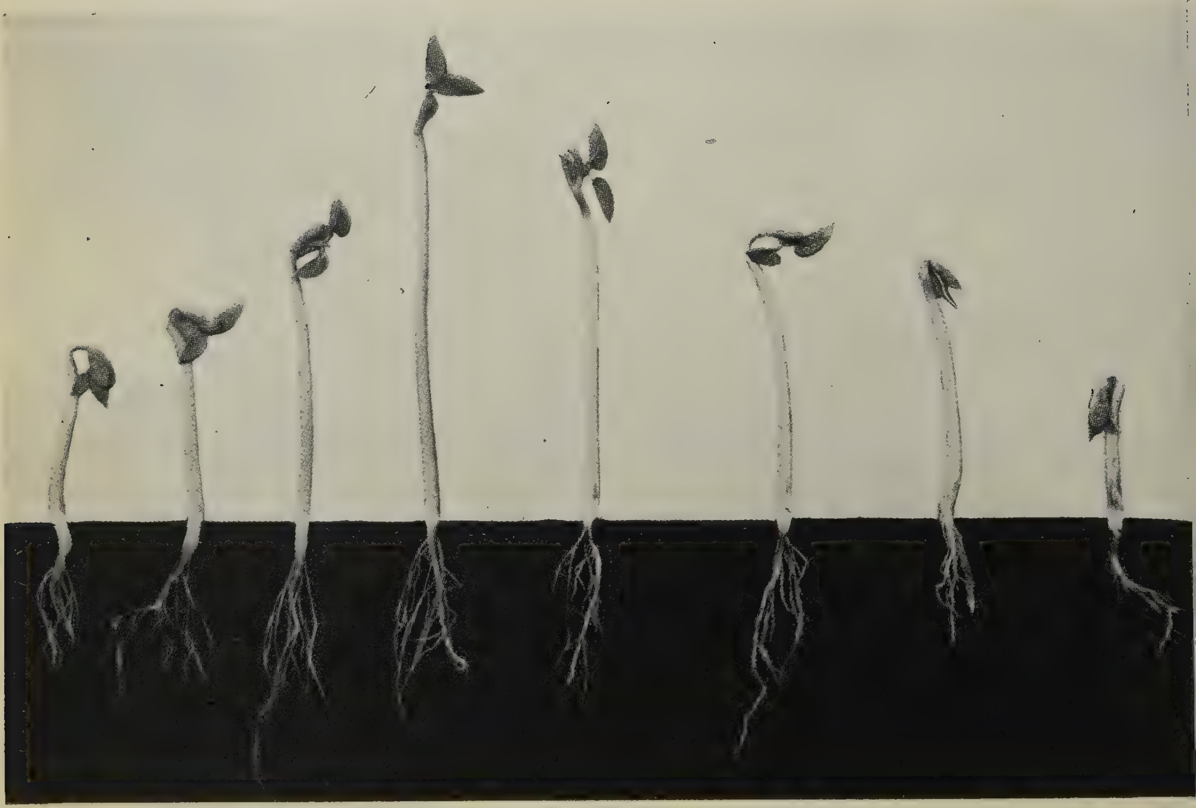
Our own observations reveal, that not only do different species exhibit different *p*H ranges for maximal growths, but even varieties have their own optimal requirements as is noticed in the various varieties of *Phaseolus vulgaris* and *Triticum vulgare*.

It is significant to note that the experimental plants show two growth maxima, frequently separated by a minimum point as in *Triticum*, *Crotalaria*, *Hordeum* and *Oryza*. In these cases the maximum values are located above *p*H 4·5—the normal acidity of Knop's solution. In *Allium* and *Phaseolus* (var. Black Altapati), however, the two maximum points lie on eitherside of the Knop's solution line, indicating a wider range of optimal *p*H, characteristic of these. These observations are in accord with the findings of Salter and McIlvaine [1917], Hixon [1920], Cole [1922], and Arrhenius [1922, 1926]. This double-maximum phenomenon as observed in the present case as well as by the above authors, calls to mind the work of Robbins [1923] who indicated that iso-electric point of proteins may be one of the factors explaining the occurrence of this phenomenon.

The optimal *p*H requirements of plants, when considered in the light of their biochemic constitutions, may be grouped in accordance with their starchy, sugary, proteinaceous and fatty nature (Table II). Plants that have a starchy nature favour a reaction from medium-acid to medium-alkali. The fatty group shows a favourable growth in the medium-acid reaction. Plants belonging to proteinaceous class, however, exhibit wider range of reactions, *viz.*, *p*H 3—6·5 (very acid to neutral).



From left to right— *p*H 3.0, 3.5, 4.4, 4.5 (Control), 5.0, 6.0, 7.0, 7.5
Effect of varying H-ions on the growth of *Cajanus indicus* (var. E.B.3 Nagpur).



From left to right—*p*H 3.0, 3.5, 4.0, 4.9, 4.5, (control) 5.0, 6.0, 7.0

Effect of varying H-ions on the growth of *Phaseolus vulgaris* (var. Red Altapati)

TABLE II

Range of optimum pH *requirement of plants according to their biochemic constitution*

Biochemic constitution	Plants experimented upon	Optimum *p*H range	Remarks
Starchy	1. *Triticum vulgare* 2. *Oryza sativa* 3. *Hordeum hexastichum*	8—4·5	Medium alkali to medium acid
Fatty	4. *Ricinus communis*	6—5	Medium acid
Sugary	5. *Allium sativum* 6. *Lycopersicum esculentum*	5—4	Acid
Proteinaceous	7. *Phaseolus vulgaris* 8. *Cicer arietinum* 9. *Cajanus indicus* 10. *Crotalaria juncea*	6·5—3	Nearly neutral to very acid

2. *Morphological characteristics under varying* pH

Apropos with growth, the morphological characteristics of plants also change in due response to *p*H of the medium. Under optimal H-ion concentrations healthy appearance of leaves and stems is observed. The roots ramify more profusely as compared to the control (grown in Knop's nutrient alone) and other *p*H values (Plates XX and XXI). With *p*H below 3·0, the roots are short and straight with little or no branches. Under increasing concentration of hydroxyl ions towards the optimum, the roots ramify to a greater extent producing a large number of lateral rootlets, some of which are so long that when projected, extend even beyond the main root tip. With a further increase in *p*H, the roots are fairly long with few branches. Addoms [1923] also noted abnormal root development side by side with the decreasing growth of plants, when grown in nutrient solutions containing relatively large quantities of potassium-di-hydrogen phosphate.

3. *H-ion changes in relation to plant growth*

The above discussion leads us to the view that plants possess the property of altering the H-ion concentration of the medium in which they grow. The change is due not to the dissolution of atmospheric carbon dioxide in the solution since variations of the buffered Knop's nutrient occur on acid as well as on alkaline side of the initial *p*H. In *Phaseolus vulgaris* (var. White Altapati), for example, the initial *p*H 3·0 is altered to 2·3, *p*H 4·9 to 3·8, *p*H 6·2 to 4·1 and *p*H 7·1 to 3·5. Stelmach [1926], Ulehla [1928], and Koslowska [1934] have also observed changes in the reaction of the medium as influenced by plants. In the present case it is found that the H-ion concentration is changed to either the acidic or alkaline side till the optimum *p*H range is attained, after which the final *p*H values exhibit no correlation with the fall in the growth. Re-stating the facts, it seems that plants can alter the H-ion concentration of the culture solution to their own requirements, provided the initial *p*H range is within a given limit. Beyond this, however, variations consequential to the growth of plants are far above or below their requirements and hence unfavourable for growth.

That there exists an intimate connection between the *p*H value and the state of the living plasma in the cell was early studied by Mevius [1927] and Arrhenius [1926]. The physiological properties of the cell and its regulation by the protein-isoelectric point has been indicated by Loeb [1924], Robbins [1923] and Pischinger [1926], but it is not possible to definitely determine the *p*H of the living plasma, since the method offers great difficulty. The presence of numerous organic phosphates, weak organic acids and strong bases (potash, soda, etc.) suggest that the degree of buffering of saps within the living cells may be considerably and directly correlated with the power of the plant to react on the medium. The so-called buffer-index curve has also been applied in physiological works by Ingold [1929] and Armstrong [1930]. When the degree of buffering (Table IV) as observed in the case of *Triticum* after the method of Koslowska [1934], is plotted against the final *p*H of the medium (fig. 1), a close parallelism is noted between the two, pointing thereby that the power of the plants to change the reaction of the medium depends to a great extent upon the degree of buffering of the sap (plant extract). The alteration in the initial *p*H of the solution which was continuously aerated, even after a few hours of plant-growth, further indicates the change to be due to causes other than the dissolution of carbon dioxide. After the lapse of a few hours the plants seem to adjust the reaction of the medium to their specific requirements. In every case the reaction is uniformly affected by a shift in the H-ion concentration to the optimum value as is further substantiated by the observation of Weis [1919] on *Zea Mays*.

TABLE III

Initial p*H*, *final* p*H*, *growth* (*linear*) *of root and shoot of different crop plants*

No. cultures	Initial *p*H	*Triticum vulgare*			*Cajanus indicus*			*Oryza sativa*		
		Final *p*H	Root growth (mm.)	Shoot growth (mm.)	Final *p*H	Root growth (mm.)	Shoot growth (mm.)	Final *p*H	Root growth (mm.)	Shoot growth (mm.)
1	3·0	2·8	No appreciable growth	178	2·6	Slight growth	No appreciable growth	3·65	No appreciable growth	No growth
2	3·1	2·8	,,	179	2·6	,,	,,	3·6	,,	,,
3	3·1	2·8	,,	180	2·7	,,	,,	3·6	,,	,,
4	3·2	2·9	,,	183	2·9	,,	,,	3·6	,,	,,
5	3·3	3·0	,,	185	2·8	,,	,,	3·62	,,	,,
6	3·3	3·0	,,	244	2·8	,,	10	3·75	,,	2·1
7	3·3	3·2	,,	245	3·0	,,	No appreciable growth	3·8	,,	3
8	3·32	3·2	,,	246	2·7	,,	,,	4·0	1·2	9
9	3·4	3·4	0·8	264	3·0	,,	,,	4·1	2·1	11
10	3·5	3·9	3·2	285	2·9	,,	,,	4·1	3·5	14
11	3·6	3·7	2·6	236	3·4	,,	,,	4·3	4	34
12	3·7	3·8	15	269	3·1	11·1	10	4·6	12	36
13	3·8	4·4	45	300	3·8	12·7	21	4·6	22	34
14	3·9	4·0	58	275	4·0	39	29	4·7	14	30

TABLE III—*contd.*

Initial pH, *final* pH, *growth* (*linear*) *of root and shoot of different crop plants*—contd.

No. cultures	Initial *p*H	*Triticum vulgare*			*Cajanus indicus*			*Oryza sativa*		
		Final *p*H	Root growth (mm.)	Shoot growth (mm.)	Final *p*H	Root growth (mm.)	Shoot growth (mm.)	Final *p*H	Root growth (mm.)	Shoot growth (mm.)
15	4·5	3·5	34	234	4·6	41	42	5·1	18	33
16	4·56	4·7	47	269	4·7	44	39	4·6	26	38
17	4·6	5·8	49	306	5·4	48	46	4·78	14	36
18	4·72	5·7	57	304	6·1	35	42	4·81	20	42
19	4·83	6·1	67	344	5·9	34	36	4·9	18	46
20	4·90	6·4	80	349	5·6	18	31	5·0	22	44
21	4·95	6·4	73	321	4·5	32	46	5·3	30	52
22	5·0	5·9	56	309	5·9	42	51	5·4	16	54
23	5·2	6·3	69	311	6·5	40	47	5·7	26	57
24	5·4	6·5	76	332	6·6	32	46	5·5	26	55
25	5·9	6·5	75	321	6·68	30	36	6·1	30	61
26	6·1	6·5	65	306	6·5	22	41	6·2	24	64
27	6·2	6·6	60	291	6·7	21	35	6·2	34	68
28	6·8	6·6	60	290	6·7	19	34	7·9	40	82
29	7.1	6·7	39	276	6·8	17	30	6·8	54	62
30	7·5	6·8	29	272	6·8			6·9	48	76
31	7·9							8·0	49	44
32	8·1							8·2	32	23
33	8·5							8·2	18	22

TABLE III—*contd.*

Initial pH, *final* pH, *growth (linear) of root and shoot of different crop plants*—contd.

No. cultures	Initial *p*H	*Allium sativum*			*Lycopersicum esculentum*			*Phaseolus vulgaris* (white altapati)		
		Final *p*H	Root growth (mm.)	Shoot growth (mm.)	Final *p*H	Root growth (mm.)	Shoot growth (mm.)	Final *p*H	Root growth (mm.)	Shoot growth (mm.)
1	3·0	3·1	No growth	3	2·6	No growth	No growth	2·3	No appreciable growth	No growth
2	3·1	3·18	,,	17	2·6	,,	,,	2·0	,,	,,
3	3·1	3·2	,,	23	2·7	,,	,,	2·1	,,	,,
4	3·2	3·2	3	42	2·8	2	1·5	2·5	,,	8
5	3·3	3·2	10	70	2·8	6	3	2·5	,,	43
6	3·3	3·3	32	90	2·8	8	4	2·7	,,	76
7	3·3	3·4	48	107	3·1	11	9	2·8	,,	98
8	3·32	3·3	41	109	3·4	14	14	3·1	,,	102
9	3·4	3·5	50	113	3·5	20	20	3·1	,,	183
10	3·5	3·9	52	115	3·6	24	24	3·5	28	262
11	3·6	4·8	63	117	3·8	34	36	3·6	40	273
12	3·7	4·5	52	109	4·0	32	48	3·7	131	283
13	3·8	4·3	33	60	4·3	32	54	3·9	119	314
14	3·9	4·2	10	59	4·2	30	48	3·7	97	232
15	4·5	3·6	4	52	3·8	32	46	3·5	98	223
16	4·56	3·5	15	79	4·0	34	54	4·1	128	313

TABLE III—*contd.*

Initial p*H*, *final* p*H*, *growth (linear) of root and shoot of different crop plants*—contd.

No. cultures	Initial *p*H	*Allium sativum*			*Lycopersicum esculentum*			*Phaseolus vulgaris* (white altapati)		
		Final *p*H	Root growth (mm.)	Shoot growth (mm.)	Final *p*H	Root growth (mm.)	Shoot growth (mm.)	Final *p*H	Root growth (mm.)	Shoot growth (mm.)
17	4·6	3·68	23	105	3·9	34	52	3·9	89	284
18	4·62	3·8	25	141	4·4	40	60	3·5	57	272
19	4·83	4·1	29	160	4·8	46	74	4·3	93	322
20	4·90	5·1	30	198	4·6	44	70	3·8	73	317
21	4·95	4·8	20	170	4·9	50	83·6	3·8	128	292
22	5·0	4·8	12	152	4·8	50·5	78	4·0	114	301
23	5·2	4·9	13	163	4·8	48	74	4·1	102	251
24	5·4	4·9	10	129	4·7	40	66	4·1	157	313
25	5·9	5·0	17	156	5·1	38	64·5	3·7	118	285
26	6·1	5·0	13	68	5·2	30	66	4·1	91	299
27	6·2	5·1	10	60	5·4	32	62	4·1	120	292
28	6·8	5·2	9	33	5·5	20	60	3·7	101	277
29	7·1	5·28	12	29	5·7	6	44	3·5	103	233
30	7·5	5·8	6	18	5·9	No appreciable growth	18	4·4	179	327
31	7·9	6·3	4	17	6·2	,,	5	5·8	82	341
32	8·1	6·6	3	11	6·6	,,	1·8	6·2	71	296
33	8·5	6·9	No growth	2	6·9	,,		6·8	34	276

TABLE III—*contd.*

Initial p*H*, *final* p*H*, *growth* (*linear*) *of root and shoot of different crop plants*—contd.

No. cultures	Initial *p*H	*Phaseolus vulgaris* (red)			*Hordeum hexastichum*			*Cicer arietinum*		
		Final *p*H	Root growth (mm.)	Shoot growth (mm.)	Final *p*H	Root growth (mm.)	Shoot growth (mm.)	Final *p*H	Root growth (mm.)	Shoot growth (mm.)
1	3·0	2·1	Slight growth	Slight growth	2·1	No growth	No growth	2·9	Slight growth	Slight growth
2	3·1	2·1	,,	,,	2·1	,,	,,	2·9	,,	,,
3	3·1	2·4	,,	,,	2·2	,,	,,	2·9	,,	,,
4	3·2	2·4	,,	,,	2·2	,,	,,	3·1	,,	,,
5	3·3	2·7	,,	,,	2·2	,,	,,	3·1	,,	,,
6	3·3	2·7	,,	,,	2·3	,,	6	3·1	,,	,,
7	3·3	2·9	,,	36	2·3	,,	12	3·2	,,	,,
8	3·32	3·1	,,	51	2·62	,,	13	3·3	,,	,,
9	3·4	3·2	,,	54	3·0	,,	24	3·2	,,	,,
10	3·5	3·5	,,	71	3·0	,,	24	3·2	,,	,,
11	3·6	3·7	6	93	3·2	,,	42	3·4	3	11
12	3·7	3·6	3	58	3·6	3·5	66	3·4	18	10
13	3·8	3·3	2	82	4·1	6	90	3·7	7	14
14	3·9	3·5	4·5	59	4·5	12	90	3·7	2	18
15	4·5	3·7	7	96	4·6	13	93	3·6	5	12
16	4·56	3·7	10	66	4·7	15	102	3·7	9	23

TABLE III *contd.*

Initial pH, *final* pH, *growth* (*linear*) *of root and shoot of different crop plants*—contd.

No. cultures	Initial *p*H	*Phaseolus vulgaris* (red)			*Hordeum hexastichum*			*Cicer arietinum*		
		Final *p*H	Root growth (mm.)	Shoot growth (mm.)	Final *p*H	Root growth (mm.)	Shoot growth (mm.)	Final *p*H	Root growth (mm.)	Shoot growth (mm.)
17	4·6	4·1	53	79	5·0	24	120	3·7	28	23
18	4·72	4·4	55	134	5·1	18	126	4·4	24	31
19	4·83	4·2	22	97	4·9	33	120	3·9	15	22
20	4·90	4·0	34	95	5·1	33	120·5	4·2	14	14
21	4·95	4·1	49	83	5·2	24	138	3·9	20	10
22	5·0	3·9	38	121	5·3	54	180	4·2	26	18
23	5·2	3·8	40	84	5·1	30	146	4·3	17	25
24	5·4	3·6	35	89	5·2	24	120	4·1	22	12
25	5·9	3·5	34	134	5·8	66	174	4·5	28	17
26	6·1	4·2	39	138	5·9	42	150	4·2	33	16
27	6·2	4·2	25	120	5·9	30	90	4·4	21	31
28	6·8	4·9	59	141	5·95	18	66	4·8	21	27
29	7·1	5·0	22	81	6·0	18	54	4·3	27	23
30	7·5	5·2	13	79	6·1	7	42	5·4	18	39
31	7·9	5·4	33	75	6·2	No growth	39	6·2	2	23
32	8·1	5·6	6	11	6·3	,,	32	6·4	0	17
33	8·5	5·7	4	6	6·3	,,	32	6·5	0	11
34										

TABLE III—*contd.*

Initial p*H*, *final* p*H*, *growth* (*linear*) *of root and shoot of different crop plants*—contd.

No. cultures	Initial *p*H	*Crotalaria juncea*			*Ricinus communis*		
		Final *p*H	Root growth (mm.)	Shoot growth (mm.)	Final *p*H	Root growth (mm.)	Shoot growth (mm.)
1	3·0	2·7	Slight growth	Slight growth	3·7	No appreciable growth.	No appreciable growth.
2	3·1	2·7	,,	,,	3·7	,,	,,
3	3·1	2·8	,,	,,	3·7	,,	,,
4	3·2	2·9	,,	,,	3·8	,,	,,
5	3·3	2·8	,,	,,	3·8	,,	,,
6	3·3	2·8	,,	,,	3·9	,,	,,
7	3·3	2·8	,,	,,	3·8	,,	,,
8	3·32	2·9	,,	,,	3·8	,,	,,
9	3·4	2·9	,,	,,	3·9	,,	,,
10	3·5	2·9	,,	,,	3·9	,,	,,
11	3·6	3·0	,,	,,	4·1	,,	,,
12	3·7	3·0	,,	10	4·7	,,	,,
13	3·8	3·8	11·1	21	4·5	12	100
14	3·9	4·0	12·7	29	4·5	5	74
15	4·5	4·6	39	31	4·6	7	87
16	4·56	4·7	41	42	5·0	10	95

TABLE III—*concld.*

Initial p*H*, *final* p*H*, *growth* (*linear*) *of root and shoot of different crop plants*—concld.

No. cultures.	Initial *p*H	*Crotalaria juncea*			*Ricinus communis*		
		Final *p*H	Root growth (mm.)	Shoot growth (mm.)	Final *p*H	Root growth (mm.)	Shoot growth (mm.)
17	4·6	5·4	44	39	5·2	9	82
18	4·72	6·1	48	46	4·3	2	92
19	4·83	5·9	35	42	4·3	10	67
20	4·90	5·6	34	36	5·9	20	156
21	4·95	4·5	18	31	5·8	32	104
22	5·0	5·9	32	46	5·8	20	113
23	5·2	6·5	42	51	5·5	31	113
24	5·4	6·6	40	47	6·2	40	102
25	5·9	6·68	32	46	4·6	18	90
26	6·1	6·5	30	36	4·8	24	89
27	6·2	6·7	22	41	4·2	18	86
28	6·8	6·7	21	35	5·1	25	80
29	7·1	6·8	19	34	5·0	24	84
30	7·5	6·8	17	30	5·6	38	108
31	7·9				5·7	37	120
32	8·1				6·5	11	86
33	8·5				6·8	3	73

TABLE IV

N/10 acid required to bring the p*H of the plant* (*Triticum*) *extract to the* p*H of the medium* (*potentiometric titrations*)

Original *p*H of the plant extract = 6·3

No. of cultures	*p*H of the culture medium	*N*/10 HCl added to the plant extract (c.c.)	Degree of buffering
1	4·7	2·30	1·30
2	5·8	0·70	1·20
3	5·7	0·86	1·30
4	6·1	0·28	1·30
5	6·4	0·12	1·60
6	6·4	0·12	1·60
7	5·9	0·57	0·62
8	6·2	0·14	1·30
9	6·5	0·28	1·80
10	6·5	0·28	1·80
11	6·5	0·28	1·80
12	6·6	0·01	1·28
13	6·6	0·01	1·28
14	6·7	0·58	1·30

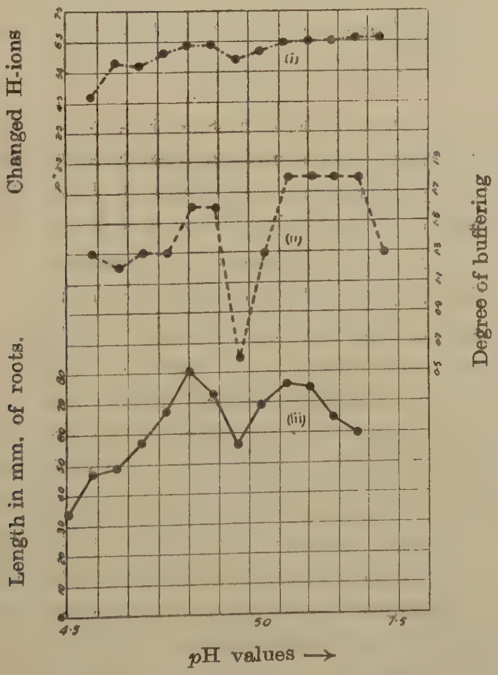


Fig. 1. *Triticum vulgare* (var. Pusa 4)
Curve (i). Changed H-ions.
Curve (ii). Degree of buffering.
Curve (iii). Linear growth.

A detailed analysis of the data further indicates that different plants bring about different reactions of the culture-solutions—a fact which has also been noted by Jacobson [1925]. In the light of these investigations it appears that the growth of plants does not depend so much on the initial *p*H of the solution as upon its degree of change to final. Jones and Shive [1922], experimenting with a number of nutrients for growing *Triticum*, recognized the buffering properties of some of them which alter only slightly. In this case, all the nutrient solutions got changed in reaction except Crone's and Sach's, the initial *p*H of which as they observed were 6·6 and 6·7 respectively. Since the initial H-ion concentrations of these solutions were in the vicinity of the range of optimum *p*H requirement of *Triticum*, as determined in the present experiments, no such change occurred. This suggests that *Triticum* has altered the reaction of these two culture-solutions by the buffering property of its cell-sap very slightly as compared to those solutions whose *p*H values are far below or above the optimal range.

Toole and Tottingham [1918], Meier and Halstead [1921], Hoagland [1923] and Parija [1934] observed that the plants growing in nutrient solutions with reactions either above or below the neutral point, tend to bring about the *p*H to neutrality. It may be reasonable to infer that these plants (*viz.*, *Triticum* sp., *Hordeum* sp. and *Eichhornia crassipes*) changed the *p*H to the optimal value which lies near neutrality.

The plants grown in the natural substratum, *viz.*, the soil at various points on the Experimental Farm, create more or less similar changes in the *p*H of the medium (Table V). The initial *p*H of the soil of all the *Triticum* plots, except No. 2, lie above *p*H 5·0—a limit from where the plants are able to adjust them according to their specific requirements. A luxuriant growth is observed in all these cases. In plot No. 2, the initial *p*H is only 4·7 (below the specific limit) which is finally changed to 5·9; the general growth of plants as a result of this is less vigorous as compared to the previous series. For the same reason in *Lycopersicum* also, good growth is noted in plots No. 1, 2, 4, 5 and 6 while in No. 3 it is found to be considerably less, because the initial *p*H of the medium lies far above the adjustable limit. The initial *p*H for *Cicer* plots are near-about the optimal *p*H requirement of the crop and hence the changes to final *p*H values are less marked. More or less similar results are obtained for other crops thus experimented upon. The final *p*H values of the medium (at harvesting time) obtained in these experiments (Table V) lie approximately within the same range as obtained for the same plants grown in the culture nutrient under controlled laboratory conditions.

TABLE V

H-ion changes in the soil from a number of representative plots of each crop

Crop	No. of plots	*p*H values of the soil		Difference between *p*H values	Difference divided by probable error	Optimum *p*H requirements
		Initial	Final			
Triticum vulgare	1	5·2±0·021	6·3±0·019	+1·1±0·028	39·2	6·4 to 6·5
	2	4·7±0·026	5·9±0·030	+1·2±0·039	30·7	
	3	5·4±0·034	6·4±0·043	+1·0±0·054	18·5	
	4	5·7±0·021	6·5±0·032	+0·8±0·038	21·0	
	5	5·6±0·018	6·4±0·025	+0·8±0·031	25·8	
	6	5·1±0·025	6·3±0·022	+1·2±0·033	36·3	
Lycopersicum esculentum	1	5·1±0·025	4·6±0·023	—0·5±0·034	14·7	4·3 to 4·9
	2	5·3±0·024	4·8±0·011	—0·5±0·026	19·2	
	3	6·2±0·032	5·2±0·015	—1·0±0·035	28·5	
	4	5·1±0·023	4·7±0·024	—0·4±0·033	12·1	
	5	6·8±0·034	4·9±0·028	—1·9±0·044	43·1	
	6	6·3±0·031	4·7±0·025	—1·6±0·039	41·0	
Cicer arietinum .	1	4·8±0·016	4·3±0·011	—0·5±0·019	26·3	4·4 to 4·8
	2	5·1±0·023	4·7±0·013	—0·4±0·026	15·3	
	3	4·7±0·024	4·4±0·014	—0·3±0·027	11·1	
	4	4·1±0·016	4·6±0·025	—0·5±0·028	17·8	
	5	5·2±0·035	4·4±0·021	—0·8±0·041	19·5	
	6	4·8±0·017	4·8±0·012	..	..	
Cajanus indicus	1	4·7±0·032	3·5±0·028	—1·2±0·043	27·9	3·3 to 4·4
	2	5·6±0·019	4·8±0·016	—0·8±0·024	33·3	
	3	5·3±0·017	4·5±0·018	—0·8±0·025	32·0	
	4	5·5±0·027	4·7±0·017	—0·8±0·031	25·8	
	5	5·7±0·030	4·8±0·012	—0·9±0·032	28·1	
	6	5·1±0·025	4·3±0·022	—0·8±0·033	24·2	
Allium sativum .	1	5·2±0·011	5·2±0·013	..	..	4·8 to 5·1
	2	3·8±0·013	4·2±0·018	+0·4±0·022	18·2	
	3	3·8±0·012	4·2±0·014	+0·4±0·018	22·2	
	4	4·8±0·011	4·9±0·012	+0·1±0·016	6·2	
	5	4·7±0·018	5·0±0·013	+0·3±0·022	13·6	
	6	3·1±0·030	4·6±0·019	+1·5±0·035	42·8	

This emphasises the importance of the initial *p*H to be adjusted prior to sowing within such a range as may ultimately be changed by the plants according to their specific requirements or else the plants may fail to grow vigorously. There is no fixity, however, in the *p*H of a particular soil for considerable changes are capable of being induced owing to plants growing on it. It seems, therefore, that there is an inter-dependence of the plant and the substratum, and it would be misleading to believe that the initial H-ion concentration of the medium controls the development of plant species, for actually it is the final one, upon which the growth and development of plants depend, except in cases where the initial

pH is too near the optimal value. A soil, if acidic in character need not necessarily require lime to bring its pH to neutrality. For such soils, the initial pH must of necessity be compared with the optimum pH requirements of the crops, and in case it is found to be far above or below the specific range, then only such a treatment be necessary. If the initial pH of the soil lies near-about the optimum pH requirement as is typically found in *Cicer* plots in our experiments (Table V), the crops growing on it will adjust the pH automatically and hence no artificial adjustment is necessary.

IV. SUMMARY AND CONCLUSIONS

In the present investigation an attempt has been made to study under controlled laboratory conditions, the growth behaviour of a number of crop plants of varying constitutions, in culture-solutions of different hydrogen-ion concentrations. The response of plants is correlated with (*i*) the degree of buffering of their saps and (*ii*) the resulting acidity of the medium, and attempt made to determining a suitable range necessary for obtaining maximum growth. Parallel studies have been made as to the hydrogen-ion drifts occurring in soil-cultures in relation to crops growing on them. The data thus amassed, are tested statistically for significance.

The following conclusions are the outcome of the above enquiry :—

Plants usually alter the pH of the growing medium ; the power of changing differs from plant to plant. The phenomenon is explainable by the analogy observed between the changed H-ions and the buffering properties of cell-sap.

The growth of plants depends to a large extent upon the ‘ final ’ hydrogen-ion concentration of the medium.

Every plant has an adjustable specific range of the initial pH, below and beyond which the plant fails to grow normally.

Short stubby-branched root-systems are generally associated with higher concentrations of hydroxyl ions.

The optimal pH requirement of plants for obtaining maximum growth differs with different species as also with varieties, the range being in accord with their biochemic constitutions.

A study of the optimal pH requirements of plants is highly commended before taking recourse to an artificial adjustment of the soil.

REFERENCES

Addoms, Ruth M. (1923). *Amer. Jour. Bot.* **10**, 211-220.

Armstrong, J. I. (1930). *Protoplasma* (Leipzig) **8**, 313.

Arrhenius, O. (1922). *Arkiv. for Botanik*, Bd. **18**, 1-54.

Arrhenius, O. (1926). "Kalkfrage, Bodenreaktion und Pflanzenwachstum," Leipzig.

Breazeale, J. F. and Leclerc, J. A. (1912). "The growth of wheat seedlings as affected by acid or alkaline conditions" (quoted from Hoagland, 1917).

Bryan, O. C. (1923). *Soil Science* **15**, 375.

Cole, A. M. (1922). "Effect of variation in H-ion concentration on the growth of seedlings" (Thesis) Univ. Mo.

Dachnowski, A. (1914). *Amer. Jour. Bot.* **1**, 412.

Davidson, O. W. and Shive, J. W. (1934). *Soil Science* **37**, 357-385.

Hartwell, B. L. and Pember, F. R. (1907). "The relation between the effects of liming and of nutrient solutions containing different amounts of acids, upon the growth of certain cereals," (quoted from Hoagland, 1917).

Hasselbach, K. A. (1912). *Biochem. Zeitochrift* **46**, 403 (1923).

Hasselbach, K. A. and Gammeltoft, S. A. (1915). *Ibidem* **68**, 206 (1923).

Heald, F. D. (1896). *Bot. Gaz.* **22**, 125.

Hixon, R. M. (1920). *Medd. Veternskapsak, Novelinstitut* **4**, 1-28.

Hoagland, D. R. (1919). *Jour. Agric. Res.* **18**, 73-117.

——————— (1923). *Cal. Agric. Expt. Stn. Tech. Paper No.* **12**.

——————— (1926). *Sci. Agric.* **6**, 141-151 ; 177-189.

Ingold, T. (1929). *Protoplasma* **6**, 51-69.

Jacobson, H. G. M. (1925). *Jour. Amer. Soc. Agron.* **17**, 577-583 ; 583-586.

Joffe, J. L. (1920). *Soil Science* **10**, 301.

Jones, L. H. and Shive, J. W. (1922). *Bot. Gaz.* **73**, 391-400.

Kahlenberg, L. and True, R. H. (1896). *Bot. Gaz.* **22**, 81.

Koslowska, Aniela (1934). *Jour. Ecol.* **22**, No. 2.

Loeb, J. (1924). "Proteins and the theory of colloidal behaviour," New York.

Lundsgaard, C. (1912). *Biochem. Zeitschrift.* **41**, 247.

Meier, H. F. A. and Halstead, C. E. (1921). *Soil Sci.* **11**, 325-353.

Mevius, W. (1927). *Naturwissenschaft und Landwirtschaft, Heft.* **11**, 153.

Mitra, S. K. and Phukan, L. N. (1929). *Agric. Jour. Ind.* **24**, 109-116.

Miyaki, K. (1914). "Ueber die Wirkung von Sauren, Alkalien and einiger Alkali auf dem Wachstum der Reispflangen," (quoted from Hoagland, 1917).

Olsen, Carsten (1923). *Compt. Rend. Lab. Carlsberg* **15**, No. 1, 1-166.

Parija, P. (1934). *Ind. Jour. Agric. Sci.* **3**, 409.

Pischinger, A. (1926). "Die Lage des Isoelektrischen Farbbarkeit. Zeits F. Zellforschung and mikroskop. Anatomie," Berlin.

Robbins, W. J. (1923). *Amer. Jour. Bot.* **10**, 412-440.

Salter, R. M. and McIlvaine, T. C. (1917). *Jour. Agric. Res.* **19**, 73-97.

Stelmach, M. (1926). *Bull. de. l' Acad. Pol. des Sciences et des Lettres* (Cracovie).

Toole, E. H. and Tottingham, W. E. (1918). *Amer. Jour. Bot.* **5**, 452-461.

Ulehla, V. (1928). *Protoplasma* (Leipzig) **3**, 469-506.

Weis, Fr. (1919). Vandkulturforsg i forskellinge Naering-soplosninger specielt til Belysming af Manganets og., Landbohjskoles Aarsskrift, p. 239.

NOTES

NOTIFICATION NO. F.-116/34/A, DATED THE 11TH NOVEMBER 1936 ISSUED BY THE GOVERNMENT OF INDIA IN THE DEPARTMENT OF EDUCATION, HEALTH AND LANDS

In continuation of this Department notification No. 360, dated the 29th February 1924, as subsequently amended, it is notified for general information that the Chief Agricultural Officer in Sind is empowered to inspect and grant certificates in respect of plants intended for export to the United Kingdom and to other countries the Governments of which require a certificate of freedom from disease to accompany consignments of plants. Arrangements have been made at the Fruit Farm, Mirpurkhas, Sind, for inspection and certification, but 10 days notice in advance has to be given by the consignors in each case.

FOURTH INTERNATIONAL GRASSLAND CONGRESS

The Fourth International Grassland Congress is to be held in Great Britain in July 1937, under the presidency of Professor R. G. Stapledon, C.B.E., M.A., Director of the Welsh Plant Breeding Station and the Imperial Bureau for Herbage Plants, Aberystwyth, Wales. The previous Congresses in this series have been held in Europe and membership was more or less confined to European members, but delegates will come to this Fourth Congress from Great Britain, the British Dominions and Colonies, U. S. A. and numerous other countries members of the International Grassland Congress Association (Central Office in Leipzig, Germany).

The paper-reading sessions will be held in Aberystwyth from July 13th to 19th, but intending participants will be able to join in a tour of centres of grassland interest and selected farms both before and after these sessions. The tour will be made partly by motor coach and partly by rail.

Delegates can choose one of the following options :—

	July
(1) Attending paper-reading sessions and local Aberystwyth tours only	13—19
(2) Assembling at Oxford, proceeding *via* selected centres of grassland interest to Aberystwyth and participating in sessions and local tours	8—19
(3) Attending paper-reading sessions and local tours and proceeding from Aberystwyth to Newcastle, and Edinburgh, where disperse	13—23
(4) Assembling at Oxford, proceeding to Aberystwyth, attending paper-reading sessions, and local tours and proceeding thence to Edinburgh, *i.e.*, the whole tour, of which options 1, 2 and 3 are only parts	8—23

The tours have been so arranged that participants will have an opportunity to see something of British grassland farming, including livestock management, over as wide a range as possible. The limits of what can be done have been set by the amount of time available, and the obvious necessity for curtailing charges to delegates.

Special addresses will be given on certain evenings during the course of the tour, when matters of general interest emanating from the tours will be dealt with. These addresses will be given at Oxford, Cirencester, Aberystwyth and Newcastle.

Approximate quotations are now given for the cost of the tours. Participants selecting option (1) will be expected to pay the Congress Fee of two pounds (sterling) but to find their own accommodation in Aberystwyth. A list of approved hotels, hostels, etc., in Aberystwyth and the neighbourhood can be obtained from the Joint Secretaries. The charges for the other three options are as follows :—

Option (*2*).—Transport, and accommodation in colleges where possible £11. Hotel accommodation can be provided at £13 per head.

Option (*3*).—Transport, and accommodation in colleges where possible £11. Hotel accommodation can be provided at £13 per head.

Option (*4*).—The complete tour. Transport, and accommodation in colleges where possible £18. Hotel accommodation can be provided at £21 per head.

Alternative rates can be quoted for those intending to provide for their own transport on tour.

As already stated the Congress Fee for the Fourth Congress is two pounds sterling, which will entitle members to attend all sessions and to receive the printed transactions, including all abstracts in advance of the Congress meetings, and any other incidental matter relating to the Congress. The Congress fee for wives accompanying members will be one pound sterling and will admit to full membership but will not entitle such members to receive a copy of the transactions.

The paper-reading sessions to be held in Aberystwyth will be divided into three plenary and two sectionalized sessions. The sectionalized sessions will deal with the following aspects of the grassland problem.

(1) Ecology (including surveys), pasture and range management (including erosion control).
(2) Seeds mixtures (including lucerne for grazing) ; legumes for use in poor pastures.
(3) Plant breeding, genetics, and seed production.
(4) Manures and fertilizers.
(5) Nutritive value of pastures ; fodder conservation.
(6) Grassland economics.

All particulars regarding the acceptance of papers and dates for receipt of abstracts and paper manuscripts may be had from the Joint Secretaries, Agricultural Buildings, Aberystwyth, Great Britain, to whom requests for the Preliminary Programme and application form for membership and all other correspondence regarding the Congress should be addressed.

BOOK NOTICES

THE EXPERIMENTAL PRODUCTION OF HAPLOIDS AND POLYPLOIDS

To-day, polyploidy is a subject of economic interest and its experimental induction a matter of first-rate importance. The Imperial Bureau of Plant Genetics, therefore, has issued a rather full account of the information available on the subject in the form of a bulletin entitled " The Experimental Production of Haploids and Polyploids " (Price 5*s*.).

Besides an extensive bibliography on the subject, the bulletin contains a concise account of the work already done and so forms a valuable guide to the available data. It is shewn that the occurrence of haploid and polyploid cells in somatic tissues may be induced by such varied agencies as chemicals, X-rays, high and low temperatures, centrifuging, decapitation of the main stem, etc. From such cells there is always the possibility of the initiation of a new plant.

The union of polyploid sex cells probably accounts for most of the polyploid plants occurring naturally and climate may have an effect on their production. As with other ploypolid cells they may be induced by changes in temperature, by the use of X-rays, narcotics, by diseases, pests, etc. Most frequently, however, the crossing of species with different chromosome numbers has been found to give haploids and polyploids among the progeny.

The list of the crosses given is long and varied, wheat and tobacco providing the greatest number of examples. The wide range of genera to which reference is made suggests that in time the experimental production of haploids and polyploids by some means or other may become merely a matter of technique. The bibliography includes nearly 300 entries and together with the subject matter, this bulletin provides a most valuable reference work for the researcher in this and allied fields.

THE SOUTH AMERICAN POTATOES AND THEIR BREEDING VALUE

The exploitation by the Russian breeders of material gathered by their expeditions to South and Central America has given a great stimulus to potato breeding and has aroused world-wide interest. In place of the one species of cultivated potato previously known over a dozen locally cultivated species have been found, together with over thirty wild species. The results of the work on this material have been published mainly in Russian and the bulletin issued by the Imperial Bureau of Plant Genetics on "The South American Potatoes and Their Breeding Value" (Price 3*s*. 6*d*.) can therefore be considered as the first comprehensive account in English of one of the most remarkable discoveries that have ever been made in plant breeding. It deals with the collection of the material, its systematics and cytology, the origin and early history of the domestic potato and with certain specific applications of the new forms, such as their value in breeding for frost resistance and blight resistance, the use of the new species *Solanum andigenum* in breeding, and breeding for higher protein content or for short dormancy.

The work reported represents an important application of Vavilov's geographical method, with intensive study of the cytological and physiological aspects of the material and it will be read with interest not only by potato specialists but by all those who are concerned with the application of modern research methods to the improvement of economic plants.

RECENT PUBLICATIONS OF THE IMPERIAL AGRICULTURAL BUREAUX

I. OBTAINABLE FROM THE IMPERIAL BUREAU OF SOIL SCIENCE, ROTHAMSTED EXPERIMENTAL STATION, HARPENDEN, HERTS

Periodical Abstracts

Lists of publications and papers on Soil Science published in the Empire Overseas in :—

	Price	
	s.	d.
1933	1	0
1934	1	0
Soil Research in the British Empire published during 1935	1	0
Lists of Publications relating to Soils and Fertilisers—		
Published monthly, per annum, post free	10	0
Monthly Letters—		
Free to recipients, within the British Empire, of "Publications relating to Soils and Fertilisers." Subscription, outside the Empire, per annum	4	0
Recent Developments in Soil Analysis—		
Quarterly Supplement to the above publications. Separate copies each	0	6

Occasional Papers

	s.	d.
Technical Communications—		
32. Tea Soils (by H. H. Mann)	2	0
33. Organic Manures (by S. H. Jenkins)	2	0
34. Tropical Soils in relation to Tropical Crops	2	6
Annual Report : For the year 1933-34	0	6
Annual Report : For the year 1934-35	0	6
Bibliographies—		
Bibliography on Coffee	2	0
Catalogue of Journals and Periodicals in the Library of Rothamsted Experimental Station	2	0
Special Publication—		
The Katamorphism of Igneous Rocks under Humid Tropical Conditions (by the late Sir J. B. Harrison)	5	0
Bibliography of Soil Science, Fertilizers and General Agronomy, 1931-34	25	0

II. OBTAINABLE FROM THE IMPERIAL BUREAU OF ANIMAL HEALTH, VETERINARY RESEARCH LABORATORY, NEW HAW, WEYBRIDGE, SURREY

Abstracting Journal

	s.	d.
The Veterinary Bulletin—		
1931. Vol. 1. Quarterly (1st Number, April)	7	6
Annual Subscription	20	0
Subsequent Volumes. Monthly (1st Number, January)	5	0
Annual Subscription (postage paid)	40	0

Indexing Publication

	Price s.	d.
Index Veterinarius—Four issues a year. First issue April 1933—Annual Subscription (postage paid). Volumes I to III mimeographed, Volume IV onwards printed	100	0

III. OBTAINABLE FROM THE IMPERIAL BUREAU OF ANIMAL NUTRITION, ROWETT RESEARCH INSTITUTE, BUCKSBURN, ABERDEEN

Journal

	s.	d.
Nutrition Abstracts and Reviews. (Issued under the direction of the Imperial Agricultural Bureaux Council, the Medical Research Council and the Reid Library)—		
Subscription per Volume of 4 numbers	42	0
Per single number	13	0

Occasional Papers

	s.	d.
Technical Communications—		
6. The Composition of Certain African Foods and Feeding Stuffs	1	0
7. Wheat : Pre-eminence as a Cereal Food, Nutritive Value ; Relation to Health and Disease	1	0
Occasional Communications—		
1. The Effect of Climate on the Composition of Pasture Plants		

IV. OBTAINABLE FROM THE IMPERIAL BUREAU OF PLANT GENETICS (FOR CROPS OTHER THAN HERBAGE), PLANT BREEDING INSTITUTE, SCHOOL OF AGRICULTURE, CAMBRIDGE

Journal

	s.	d.
Plant Breeding Abstracts—		
Quarterly. Annual Subscription	15	0
Single copies	5	0

Occasional Papers

	s.	d.
Indexes to Plant Breeding Abstracts—		
Subject Index to Vols. I to V of Plant Breeding Abstracts	5	0
Supplements to Plant Breeding Abstracts—		
Summary of Reports received from Countries exclusive of the British Empire, 1928-31. Supplement I	2	6
Summary of Reports received from Stations in the British Empire, 1932-1935. Supplement II	5	0
Technical Communications—		
Vernalization and Phasic Development of Plants (Joint Publication of the Imperial Bureaux of Plant Genetics)	10	0
Bibliographical Monographs—		
The Experimental Production of Haploids and Polyploids	5	0
Breeding Resistant Varieties, 1930-33 (Supplement)	2	0

V. Obtainable from the Imperial Bureau of Plant Genetics (Herbage Plants), Welsh Plant Breeding Station, Agricultural Buildings, Alexandra Road, Aberystwyth, Wales

Journals

	Price s.	d.
Herbage Abstracts—		
Quarterly. Annual Subscription	15	0
Single numbers	5	0
Herbage Reviews—		
Subscription is at present—Vol. 1 (1933), Vol. 2 (1934), Vol. 3 (1935), Vol. 4 (1936)—included in that to Herbage Abstracts		

Occasional Papers

	s.	d.
Bulletins—		
15. Grassland and Forage Crops in Thuringia, Czechoslovakia and Hungary, August, 1934	3	6
17. Vernalization and Phasic Development of Plants. December, 1935. (Joint publication of the Imperial Bureaux of Plant Genetics)	10	0

VI. Obtainable from the Imperial Bureau of Fruit Production, East Malling Research Station, East Malling, Kent

Journal

	s.	d.
Horticultural Abstracts—		
A quarterly abstract publication of current horticultural literature—		
Annual Subscription	15	0
Single copy	4	0

Technical Communictions

	s.	d.
6. The Nutrition and Manuring of Soft Fruits. 1936. T. Wallace.	2	0
7. Vegetative Propagation of Tropical and Sub-tropical Fruits. 1936. J. St. Clair Feilden and R. J. Garner	2	0

Occasional Papers

	s.	d.
3. Annotated Bibliography on Bitter-Pit. 1934	1	6
Other Publications—		
Index to Volumes I-X of the *Journal of Pomology and Horticultural Science*, 1933. Compiled by Bureau, published by the Editors of the *Journal of Pomology and Horticultural Science*. Available from the Bureau	5	0
Old and New standpoints on senile degeneration, 1931. By A. P. C. Bijhouwer	0	6

VII. Obtainable from the Imperial Bureau of Animal Genetics, Institute of Animal Genetics, University of Edinburgh, King's Buildings, West Mains Road, Edinburgh

Journal

	s.	d.
Animal Breeding Abstracts (Quarterly) Commencing April, 1933. Annual Subscription	15	0

Occasional Papers

	Price s.	d.
The Technique of Artificial Insemination, 1933	2	6
Bibliography of the works of J. C. Ewart (free to subscribers of Animal Breeding Abstracts, Vol. 1), 1934	0	6
Animal Breeding in the British Empire. A Survey of Research and Experiment, 1934	2	0

VIII. OBTAINABLE FROM THE IMPERIAL BUREAU OF AGRICULTURAL PARASITOLOGY, INSTITUTE OF AGRICULTURAL PARASITOLOGY, WINCHES FARM DRIVE, HATFIELD ROAD, ST. ALBANS, HERTS

Journals

	s.	d.
Bibliography of Helminthology. For the year 1933—		
Library Edition, bound in cloth	10	6
Stiff paper cover only	8	0
Helminthological Abstracts. (Issued in five or six parts per volume, each volume, covering the literature of a single year.) Subscription per volume, payable in advance	30	0
Completed volumes bound in cloth	32	0

Occasional Papers

	s.	d.
(*g*) The Pathology and Aetiology of Plant Lesions caused by Parasitic Nematodes. By. T. Goodey, 34 pp.	5	0
(*h*) The Bearing of the Physiology of Parasitic Nematodes on their Treatment and Control. By A. Lapage. 21 pp.	3	0
Notes and Memoranda—		
11. Recent Developments in the Control of *Heterodera marioni*, 6 pp.	1	6

PUBLICATIONS ISSUED BY THE IMPERIAL INSTITUTE OF ENTOMOLOGY, 41, QUEEN'S GATE, LONDON, S. W. 7

Bulletin of Entomological Research.—Published quarterly and containing original articles on Economic Entomology. Issued post-free.

	s.	d.
Annual subscription (payable in advance)	30	0

Review of Applied Entomology.—Consisting of abstracts or reviews of all current literature on Economic Entomology throughout the world. Published monthly in two series. Series 'A' dealing with Insect and other Arthropod pests of cultivated plants, forest trees and stored products of animal and vegetable origin, Series 'B' dealing with insects, ticks, etc., conveying disease or otherwise injurious to man and animals. Issued post-free.

	Series 'A' s.	'B' s.
Annual subscription (payable in advance)	30	15
Vol. XXIII (1935)	42	21

	Price	
	s.	d.
Zoological Record—Part Insecta.—Published annually about July in each year and containing as complete a record as possible of the literature of the previous year chiefly from the systematic standpoint.		
Annual subscription (including postage)	15	6
Report of the Fourth Imperial Entomological Conference, 19-27th September 1935	4	0

Publications obtainable from the Imperial Mycological Institute, Kew, Surrey

Journal

Review of Applied Mycology.—Annual subscription, 12 monthly parts, with title page and index (post-free)	21	0
Single part	2	0
Title page and index	3	0
Report on the Third Imperial Mycological Conference, 1934	2	0

PUBLICATIONS OF THE IMPERIAL COUNCIL OF AGRICULTURAL RESEARCH, INDIA

1. Agriculture and Live-stock in India

A bi-monthly Journal of Agriculture and Animal Husbandry for the general reader interested in Agriculture or Live-stock in India or the Tropics. (Established 1931. Published in January, March, May, July, September and November. Prepayable subscription Rs. 6 or 9*s*. 9*d*. per annum inclusive of Indian postage. Price per part Rs. 2 or 3*s*. 6*d*. inclusive of Indian postage). Volumes I to VI complete are available.

2. The Indian Journal of Agricultural Science

A bi-monthly Scientific Journal of Agriculture and the allied sciences, mainly devoted to the publication of the results of original research and field experiments. (Established 1931. Published in February, April, June, August, October and December. Prepayable subscription Rs. 15 or 24*s*. per annum inclusive of Indian postage. Price per part Rs. 3 or 5*s*. 3*d*. inclusive of Indian postage.) Volumes I to VI complete are available.

3. The Indian Journal of Veterinary Science and Animal Husbandry

A quarterly Journal for the publication of scientific matter relating to the health, nutrition and breeding of live-stock. (Established 1931. Published in March, June, September and December. Prepayable subscription Rs. 6 or 9*s*. 9*d*. inclusive of Indian postage. Price per part Rs. 2 or 3*s*. 6*d*. inclusive of Indian postage.) Volumes I to VI complete are available.

4. Scientific Monographs of the Imperial Council of Agricultural Research

NCAG—SM/1	No. 1.	The Fungi of India, By E. J. Butler, C.I.E., D.Sc., M.B., F.R.S. and G. R. Bisby, Ph.D. (1931). Price Rs. 6-12-0 or 11*s*. (As. 8.)
NCAG—SM/2	No. 2.	Life-histories of Indian Microlepidoptera, Second Series: Alucitidae (Pterophoridae, Tortricina and Gelechiadae), By T. Bainbrigge Fletcher, R.N., F.L.S., F.E.S., F.Z.S. (1932). Price Rs. 3-4-0 or 5*s*. 6*d*. (As. 6.)
NCAG—SM/3	No. 3.	The Open Pan System of White- Sugar Manufacture, By R. C. Srivastava, B.Sc. (1935), (2nd edition). Price Rs. 3-2-0 or 5*s*. 6*d*. (As. 9.)
NCAG—SM/4	No. 4.	Life-histories of Indian Microlepidoptera: Cosmopterygidae to Neopseustidae, By T. Bainbrigge Fletcher, R.N., F.L.S., F.E.S., F.Z.S. (1933). Price Rs. 4-8-0 or 7*s*. 6*d*. (As. 8.)
NCAG—SM/5	No. 5.	The Bombay Grasses, By E. J. Blatter, S.J., Ph.D., F.L.S., and C. McCann, F.L.S. Illustrated by R. K. Bhide. Price Rs. 20-12-0 or 32*s*. 6*d*.
NCAG—SM/6	No. 6.	Helminth Parasites of the Domesticated Animals in India, by G. D. Bhalerao, M.Sc. Price Rs. 7-12-0 or 13*s*. 3*d*.
NCAG—SM/7	No. 7.	Influence of Manures on the Wilt Disease of *Cajanus indicus* Spreng. and the Isolation of Types Resistant to the Disease. By W. McRae, M.A., D.Sc. (Edin.), F.L.S. and F. J. F. Shaw, D.Sc. (Lond.), A.R.C.S., F.L.S. (1933). Price Rs. 2-4-0 or 4*s*. 3*d*. (As. 5.)

NCAG—SM/8	No. 8. The Silk Industry of Japan with Notes on Observations in the United States of America, England, France and Italy, By C. C. Ghosh, B.A., F.E.S. (1933). Price Rs. 4 or 6*s*. 9*d*. (As. 7.)
NCAG—SM/9	No. 9. Mechanical Cultivation in India. A History of the Large Scale Experiments carried out by Burmah-Shell Oil Storage and Distributing Company of India, Ltd., By C. P. G. Wade, Price Rs. 3-14-0 or 6*s*. 6*d*.
NCAR—SM/10	No. 10. Spotted Boll-worms of Cotton (*Earias fabia* Stoll and *Earias insulana* Boid.) in South Gujarat, (Final report on investigations financed by the Indian Central Cotton Committee. 1923-1931), By B. P. Deshpande, M.Ag. and N. T. Nandkarny. Price Rs. 5-14-0 or 9*s*. 6*d*.
NCAG—SM/11	No. 11. Investigations on the Course and Distribution of the Nerves supplying Levator anguli scapuli and Rhomboideus Muscles and the Formation of the Phrenic Nerve in the Ox, with Observations on certain Anatomical Deviations, by H. N. Chelva Ayyangar, G.M.V.C. (*In the Press*).

5. Miscellaneous Bulletins of the Imperial Council of Agricultural Research

NCAG—MB/1	No. 1. List of Publications on Indian Entomology (1930), Compiled by the Imperial Entomologist, Pusa. Price As. 14 or 1*s*. 6*d*.
NCAG—MB/2	No. 2. List of Publications on Indian Entomology (1931), Compiled by the Imperial Entomologist, Pusa. Price As. 8 or 10*d*.
NCAG—MB/3	No. 3. List of Publications on Indian Entomology (1932), Compiled by the Imperial Entomologist, Pusa. Price As. 12 or 1*s*. 3*d*.
NCAG—MB/4	No. 4. Host Plant Index of Indo-Ceylonese Coccidae, By S. Ramachandran, L.Ag. and T. V. Ramakrishna Ayyar, B.A., Ph.D., F.Z.S. Price Rs. 1-10 or 2*s*. 9*d*.
NCAG—MB/5	No. 5. List of Publications on Indian Entomology (1933), Compiled by the Officiating Imperial Entomologist, Pusa. Price As. 9 or 1*s*.
NCAG—MB/6	No. 6. Bee-keeping, By C. C. Ghosh, B.A., F.E.S. (3rd Edition.) Price Rs. 1-14 or 3*s*. 3*d*.
NCAG—MB/7	No. 7. List of Publications on Indian Entomology (1934), Compiled by the Officiating Imperial Entomologist and the Imperial Entomologist, Pusa. Price Rs. 1-2-0 or 2*s*.
NCAR—MB/8	No. 8. Selected Clinical Articles, by M. G. K. Sharma, G. P. V. C. and R. L. Kaura, B.V.Sc., M.R.C.V.S. Price As. 8 or 10*d*.
NCAR—MB/9	No. 9. Statistical Methods and their Application to Agronomy: A Bibliography, by K. K. Guha Roy, B.A. Price Rs. 2-2 or 4*s*.
NCAR—MB/10	No. 10. Diseases of Sugarcane and Methods for their Control, by L. S. Subramaniam. Price Rs. 1-14-0 or 3*s*. 3*d*.
NCAR—MB/11	No. 11. Tables of Standard Errors of Mendelian Ratios, By Swarn Singh Purewal, and P. Krishna Rao. Price As. 12 or 1*s*. 3*d*.
NCAR—MB/12	No. 12. List of Publications on the Botany of Indian Crops, Part II, for the period 1928-32, by R. D. Bose. Price Rs. 3-6-0 or 5*s*. 9*d*.
NCAR—MB/13	No. 13. Two New Statistical Tables based upon Fisher's '*t*', by M. Vaidyanathan. Price As. 6 or 8*d*.
NCAR—MB/14	No. 14.—List of Publications on Indian Entomology (1935), Compiled by the Imperial Entomologist. (*In the Press*).

6. Annual Report of the Imperial Council of Agricultural Research.

NCAG—AR/31	Annual Report of the Imperial Council of Agricultural Research for the years 1929-30 and 1930-31. Price As. 12 or 1*s.* 3*d.* (Anna 1.)
NCAG—AR/32	Annual Report of the Imperial Council of Agricultural Research for the year 1931-32. Price As. 6 or 8*d.* (As. 2).
NCAG—AR/33	Annual Report of the Imperial Council of Agricultural Research for the year 1932-33. Price As. 6 or 8*d.*
NCAG—AR/34	Annual Report of the Imperial Council of Agricultural Research for the year 1933-34.
NCAG—AR/35	Annual Report of the Imperial Council of Agricultural Research for the year 1934-35.
NCAR—AR/36	Annual Report of the Imperial Council of Agricultural Research for the year 1935-36.

7. Agriculture and Animal Husbandry in India (formerly called Review of Agricultural Operations in India).

NCAG—R. 1/29	Review of Agricultural Operations in India, 1928-29. Price Rs. 3-2-0 or 5*s.* 6*d.* (As. 8.)
NCAG—R. 1/31	Review of Agricultural Operations in India, 1929-31. Price Rs. 5 or 8*s.* 3*d.* (As. 10).
NCAG—R. 1/33	Review of Agricultural Operations in India, 1931-33. Price Rs. 5-12-0 or 9*s.* 6*d.*
NCAG—R. 1/35	Agriculture and Animal Husbandry in India : Part I—Crop Production. Price Rs. 4-14-0 or 8*s.* Part II—Animal Husbandry. Price Re. 1 or 1*s.* 9*d.*

8. Proceedings of the Board of Agriculture and Animal Husbandry.

NCAG—P. 1	Proceedings of the First Meeting of the Animal Husbandry Wing of the Board of Agriculture and Animal Husbandry held at New Delhi from the 20th to 23rd February 1933, with Appendices. Price Rs. 5-14-0 or 9*s.* 6*d.*
NCAR—P. 2	Proceedings of the First Meeting of the Crops and Soils Wing of the Board of Agriculture and Animal Husbandry in India held at Delhi from the 25th February to the 2nd March 1935, with Appendices. Price Rs. 6 or 9*s.* 9*d.*

A description of the Imperial Institute of Veterinary Research, Muktesar, and its Sub-station, the Imperial Veterinary Serum Institute, Izatnagar, by F. Ware, F.R.C.V.S. Price Rs. 1-4-0 or 2*s.*

The Production of Cigarette Tobacco by Flue-curing, by F. J. F. Shaw, C.I.E., D.Sc., A.R.C.S., F.L.S. and Kashi Ram. *Imp. Inst. Agri. Pes. Pusa Bull.* No. 187. Reprinted (1935). Price Re. 1 or 1*s.* 9*d.*

A Handbook of statistics for use in Plant Breeding and Agricultural Problems, By F. J. F. Shaw, C.I.E., D.Sc., A.R.C.S., F.L.S. Price Rs. 4-6-0 or 7*s.* 3*d.*

[Copies of the above publications can be had from :—

The Manager of Publications, Civil Lines, Delhi, India.

In Europe and America.—From Public Department (Publications Branch), Office of the High Commissioner for India, India House, Aldwych, London, W. C. 2.]

GIPD—664 ICofAR—5-5-37—800.

ORIGINAL ARTICLES

TYPES OF TOBACCO LEAF-CURL IN NORTHERN INDIA

BY
B. P. PAL, M.Sc., Ph.D. (Cantab.), F.L.S.
Imperial Economic Botanist
AND
R. K. TANDON, M.Sc.
Post-graduate Student, Imperial Agricultural Research Institute, New Delhi.

(Received for publication on 11th November 1936)

(With Plates XXIIX-XXI, and five text-figures)

CONTENTS

PAGE.
. . 363

ERRATA.

Vol. 7, Part III, June 1937.

Page 381, line 10 from bottom *for* ' ofl eaf-curl ' *read* ' of leaf-curl '.

Page 381, line 3 from bottom *for* ' this usually more pronounced ' *read* ' this is usually more pronounced '.

Plate XLVII (facing page 513), letterpress.—*for* ' Bambusa arundinace ' *read* ' Bambusa arundinacea '.

diseases described by some writers. It is also 'frenching ' although it has now been established [Valleau and Johnson, 1927; etc.] that true ' frenching ' is a distinct abnormality brought about by non-pathological causes. Leaf-curl is a menace to tobacco cultivation in Northern India, East Africa, Rhodesia, and Java, and has also been reported in the U. S. S. R. [Khudyna, 1936]. It has not, however, been studied nearly so extensively as the mosaic diseases of tobacco and this is in all probability to be attributed to the fact that leaf-curl is apparently entirely absent in those countries, *e.g* , the United States, where a major portion of the work on tobacco viruses has been done.

With the development of the cigarette tobacco industry in India and the increase in the area under ' bright ' tobaccos, the seriousness of the damage caused by leaf-curl has received greater recognition. For while the incidence of this group of diseases reduced the yield, and to some extent the quality, of the coarse dark types previously grown, diseased plants could still be utilised for the preparation of chewing tobacco, snuff, etc., and were not a total loss. With tobaccos intended for cigarette manufacture, however, the position is quite different. The leaf for this purpose must be thin, smooth and of fine texture and should cure to a uniform lemon-yellow colour. The leaves of a ' curled ' plant are almost invariably twisted, ruffled, puckered and thickened, with abnormally prominent veins, and are absolutely useless for the flue-curing barn. When it is mentioned that in an epidemic year over ninety per cent of the crop may be infected, the heavy toll taken by leaf-curl is apparent and it is needless to point out the necessity for work to investigate these diseases and to devise measures of control.

Leaf-curl is widespread throughout the tobacco-growing area in the North Indian plain. Plants exhibiting symptoms similar to those of leaf-curl were seen recently at Poona by Dr. B. B. Mundkur and it is possible that the leaf-curl diseases are also present in Western India. But they appear to be rare or absent in the Guntur District of the Madras Presidency where the largest quantity of cigarette tobacco is grown. Tobacco mosaic is also common in India but is relatively unimportant.

The investigations reported in this paper were commenced at the suggestion of Dr. F. J. F. Shaw in the latter part of 1933 when the senior author was appointed at Pusa, and were carried out in collaboration with those in progress in the Entomological Section. The results of the latter will be published in a subsequent issue of this *Journal* and the two papers should be read together. The writers wish to take this opportunity to express their appreciation of the Imperial Entomologist's co-operation in this project.

Very little was then known about the diseases and it became necessary, first of all, to determine whether the leaf-curl in India was a virus disease of the kind described by Storey [1931] in Africa and by Thung [1932] in Java, or whether it was ' frenching ' due to adverse soil conditions or nutritional deficiencies. The symptoms, however, were obviously in agreement with those described by Storey [1931, 1932, 1935]. As a preliminary test, six reciprocal pairs of grafts were made, each pair consisting of a healthy scion and a diseased stock, and *vice versa*. Without exception all the originally healthy scions showed unmistakable symptoms of the disease within a month from the date of grafting whereas control grafts (healthy grafted on healthy) remained healthy. In cases where a diseased scion was grafted on a healthy stock the originally fully grown leaves of the stock showed no apparent reaction but the new growth from the base was diseased. These results indicated that leaf-curl was infectious, and of the nature of a virus disease (or group of diseases).

An interesting case of incomplete infection was noticed in a plant of tobacco at this time, approximately half the stem being healthy and the other half diseased. All the leaves and suckers on the healthy side of the stem were normal, while all those on the other side were ' curled '. Sections of the stem were cut but no obvious differences in the anatomy of the two portions were discerned and the reason why the disease was restricted to one side of the plant is not yet understood.

II. Review of literature

It is probably not possible to establish with any certainty the first record of leaf-curl in tobacco. As Storey [1932] has pointed out, leaf-curling which is one of the characteristics of this group of diseases may also be brought about by several other agencies, such as aphid infestation, and at least one abnormality of this kind is inherited. Again Roberts [1930] concluded that a ' crinkle ' could be caused in tobacco by the poisonous nature of the saliva of a capsid, *Engytatus volucer* Kirk. As a result, it is usually difficult to distinguish true leaf-curl from conditions which simulate it as described in the writings of some of the earlier workers in this field. It is quite clear, however, from the description and the illustrations given by Ludwig [1913] that the ' kroepoek ' disease reported by him in the Cameroons is the same as leaf-curl. Thung [1932] also is of opinion that the ' faltenzwerg ', ' gila ', ' krekoh ', etc., of earlier writers are identical with the ' kroepoek ' and ' krulziekte ' described by him.

The real nature of leaf-curl however was not recognised until Storey, in 1931, demonstrated that it was due to a virus which was transmitted by a species of white-fly. This was confirmed by Thung [1932]. The disease as it occurs in South and East Africa has been described by Storey [1932], Hopkins [1932], Hornby [1932], etc., and the present position has been excellently summarised by Storey [1935]. Although these investigators have noted considerable variation in the symptoms, and Storey [1935] has referred to the possibility of differences in the virulence of strains of the virus, no attempt has been made by the African workers to distinguish different types of the disease. Thung [1932] however differentiates three distinct types in Java :—

(*a*) The ' gewone kroepoek ' characterised by curling of the leaves, thickening of the veins, and the presence of enations on the underside of the leaf.

(*b*) The ' krulziekte ' characterised by more severe curling of the leaf edge, rugosity, and the presence of enations.

(*c*) The ' heldere kroepoek ' characterised by rolling of the leaves and vein-clearing, both thickening of the veins and enations being absent.

The anatomy of these types has been studied by Thung himself [1932] and by Kerling [1933], the latter using material provided by Thung.

In India the occurrence of leaf-curl in tobacco has been briefly noted in the reports of the Imperial Economic Botanist [1926], etc. Other detailed published information does not appear to be available. No other record of the disease in this country has come to the writers' notice.

III. Material and method

Varieties.—The symptoms exhibited by a virus on a host may vary greatly with the variety and the question of variety is very important in virus disease investigations. The greater portion of the work reported in this paper was therefore carried out with one variety of tobacco, *viz.*, Pusa H 142. This belongs to the 'bright' or 'Virginia' group of tobaccos and its origin and description have been given by Kashi Ram [1931].

The other varieties used or to which reference is made are :

N. Tabacum.—Cigarette tobacco group :—

- Pusa H 177
- Adcock
- Harrison's Special
- Cash
- Connecticut Havana No. 38
- Cavalla
- Turkish

Chewing tobacco group :—

- Pusa T 28
- ,, T 58
- ,, T 63

N. rustica.—*Hookah* tobacco :—

- Pusa T 18 [Howard and Howard, 1910].

The cigarette group is characterised by smooth, thin leaves, the chewing group by coarse, thick and uneven leaves. Connecticut Havana No. 38, a cigar tobacco, has been placed in the former group as it behaved similarly, in respect of reaction to leaf-curl viruses, to other varieties in this group. Cavalla and Turkish are very distinct from the others. *N. rustica* (Pusa T 18) has the thick, coarse rounded leaves typical of this species.

Besides the above-mentioned tobaccos, *Nicotiana glutinosa*, *Solanum nigrum*, *Lycopersicum esculentum*, and *Petunia* sp. (common garden variety), were used as differentials. Seed of Connecticut Havana No. 38 was obtained from Dr. James Johnson of Wisconsin. Seeds of *N. glutinosa* and *N. Tabacum* var. Turkish were kindly supplied by Dr. B. B. Mundkur of the Mycological Section.

Protection from insects.—Except where otherwise stated, all 'healthy' material referred to in this paper was raised under muslin or fine wire-gauze cages. Later the seedlings were transplanted into the field and immediately enclosed within muslin bags. Under such conditions not a single seedling contracted leaf-curl. In very rare cases, however, seedlings became infected with mosaic (possibly tobacco virus No. 1 of James Johnson) [Johnson, 1927]. As the mosaic virus is easily transmissible by juice and of remarkable longevity, it is perhaps hardly surprising that it should be able to spread from a diseased plant to healthy ones in the course of routine cultural operations. In one or two cases plants previously suffering from leaf-curl later showed mosaic infection. All such plants were discarded.

Grafting technique.—Grafting was achieved by inserting the scion, the stalk of which had been cut into a wedge, into a slit in the stem of the stock. Scion and stock were held in position by binding with grafting tape (bandage soaked in molten beeswax). This method was found to be highly successful. The usual precautions to secure asepsis were taken; hands and instruments were sterilised with absolute alcohol before and after every operation.

Inoculation technique.—Two methods of inoculation were used : (1) A diseased leaf was placed over a young healthy leaf and the latter pierced through the former with a set of five entomological pins mounted on a cork. Two leaves per plant were treated in this way. (2) The young leaves and shoot of a diseased plant were triturated and the solution obtained diluted with an equal quantity of water; a pad of cheese-cloth dipped in the diluted solution was then rubbed lightly but firmly on two young leaves of a healthy plant.

Age of seedlings at time of grafting and inoculation.—Inoculations were made when healthy seedlings were about eight weeks old.

In the case of grafts, the age varied according to the particular experiment. Where the age of the seedlings is not comparable, this is stated in the proper place. As far as possible young seedlings were used as those usually exhibit symptoms more clearly than mature plants; also the latter are more woody and success in grafting is not so certain with them. A limiting factor in this connection was the fact that it was not possible to distinguish between the different types of leaf-curl until the plants had reached a certain size, the initial symptoms being similar in most cases.

Effect of enclosing in bags.—All experimental plants in the field were enclosed in fine muslin bags. In the case of diseased plants, the latter were usually bagged for a full month before grafting during which period they were examined from time to time to determine whether the symptoms remained reasonably constant or whether there was any suspicion of admixture of viruses. Prolonged bagging, however, slightly distorted the symptoms in the case of the less virulent leaf-curl viruses. Plants under bag were therefore always compared with non-bagged plants of the same types and in this way due allowance could be made for the effects of bagging, in diagnosing the symptoms.

*Terms used**.—As there is a certain amount of confusion in the literature regarding the exact meaning of some of the terms commonly employed in the description of virus diseases, the meanings of the terms used in this paper are explained below :—

Curling—an abnormal downward bending of the leaf blade.

Rolling—an upward curving of the sides of each leaf so that the midrib is at the bottom of the trough so formed.

Wrinkling—an abnormal unevenness of the leaf blade due to depressions and prominences not arranged in any uniform manner as with rugosity.

Puckering—the same as wrinkling but the prominences are more marked and large.

Rugosity—like wrinkling but differs in having the depressions only at the veins and in having the prominences of uniform height.

Ruffling—an abnormal unevenness of the leaf blade surface caused by ridges that develop or become more pronounced with passage from the midrib to lateral margins, resulting in waviness of the margin.

Greening—the development in the veins of the lower surface of the same deep green colour as is found in the lamina of the upper surface. This is always accompanied by a thickening of the veins, and may be followed by the development of outgrowths or enations. In certain cases the greening is apparent only when the leaf is held against the sky and has not yet come to the surface. In such cases it is referred to as ' unburst greening '.

IV. OBSERVATIONS ON INCIDENCE AND EPIDEMIOLOGY

A certain amount of leaf-curl occurs every year at Pusa and it has been estimated, by observation alone, that the amount varies in a normal year from five to ten per cent. The crop-year 1934-35 in which investigations on leaf-curl were started in earnest, was an epidemic year and the incidence within the Botanical Area rose to as high as about seventy per cent in the case of some varieties. A part of one of the tobacco plots in that year is shown in Plate XXII, fig. 1. In the neighbouring villages, the percentage of diseased plants in the tobacco fields was even higher and in certain cases the crop was ploughed up and the land re-sown with wheat.

Nothing was known at the time the present research was begun about the factors controlling the distribution and rate of spread of the disease. The usual time of sowing tobacco is the third week in August but there was a general impression that if sowing was done earlier the amount of leaf-curl (and also of

---

* Most of these are taken from Shultz and Folsom (*Jour. Agric. Res.* **25**, 43-117 : 1923) with slight modification.

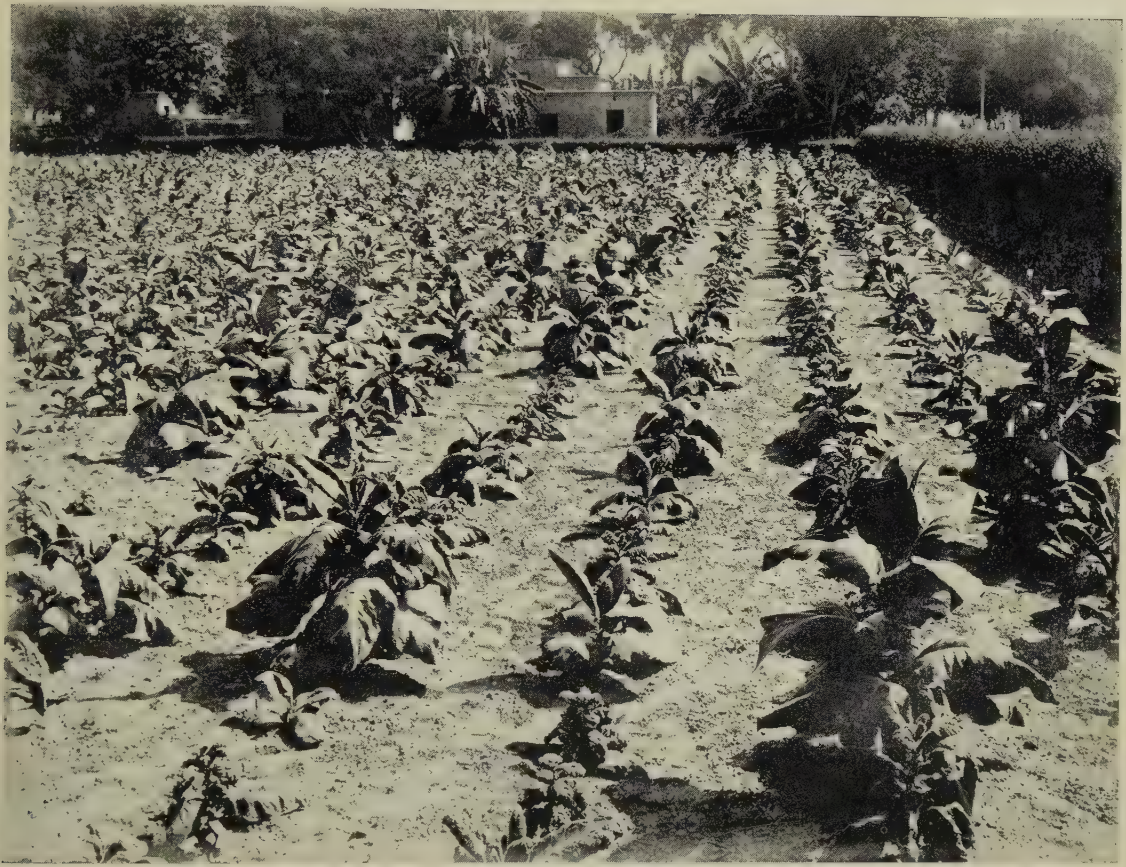


FIG. 1. Part of tobacco field in 1934-35 showing remarkably high incidence of leaf-curl. The two tall plants in the right foreground are healthy.

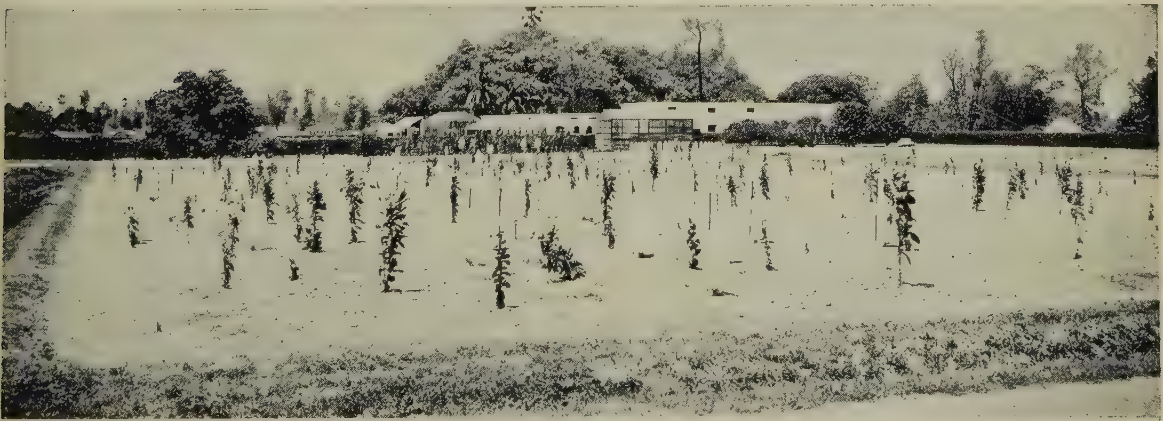


FIG. 2. Orchard 2-B tobacco plot in 1935-36 showing distribution of leaf-curl plants (healthy plants were removed before the photograph was taken).

Cercospora leaf-spot) would be greater. The correctness or otherwise of this view was tested to some extent in 1935-36 (*vide infra*).

Detailed observations for two years on the crop of Pusa H 142 and other types of tobacco covering approximately two acres which is usually grown every year in the Botanical Area are summarised in Tables I, II and III.

TABLE I

Numbers and percentages of leaf-curl plants in several varieties of tobacco in 1934-35.

Field	Variety	Total No. of plants in field	Total No. of plants including replacements	Number of leaf-curl plants *		
				1st count (October)	2nd count (November)	Total
	Cigarette tobaccos					
Musahar 2 . .	H 142 † . .	5712	6720	1008 *17·7*	2306 *40·4*	3314 *49·3*
Musahar 3 . .	H 142 † . .	5712	6765	1053 *18·4*	2338 *40·9*	3391 *50·1*
Musahar 4 . .	H 142 . .	3488	3488	1033 *29·6*	1370 *39·3*	2403 *68·9*
,, . .	Adcock . .	654	654	39 *6·0*	225 *34·4*	264 *40·4*
,, . .	Harrison's Special.	436	436	58 *13·3*	235 *53·9*	293 *67·2*
Musahar 1 . .	Cash . .	696	696	253 *36·4*	109 *15·7*	362 *52·0*
,, . .	H 17†‡ . .	754	754	..	74 *9·8*	74 *9·8*

* Figures in *Italics* are percentages.

† Rogueing was carried out.

‡ Sown late. The incidence of disease in this variety is therefore not comparable with that in other varieties.

TABLE I—*contd.*

Numbers and percentages of leaf-curl plants in several varieties of tobacco in 1934-35—contd.

Field	Variety	Total No. of plants in field	Total No. of plants including replacements	Number of leaf-curl plants*		
				1st count (October)	2nd count (November)	Total
			Chewing tobaccos			
S T G 4 . . .	T 28 † . .	464	651	178 *38·4*	122 *26·3*	300 *46·1*
	T 58 † . .	406	586	180 *44·3*	102 *15·1*	282 *48·1*
	T 63 † . .	464	720	256 *55·17*	103 *22·0*	359 *49·86*

*Figures in *Italics* are percentages.

†Rogueing was carried out.

1934-35.—Two counts of diseased plants were taken, one in the first week of October and the other a month later. The dates of sowing and planting out were 17th August and 23rd to 26th September, respectively. In the early stages diseased plants of Pusa H 142 in Musahar No. 2 and No. 3 plots were removed and replaced with healthy ones in accordance with the customary practice. In the other two plots (Musahar No. 1 and No. 4) however no rogueing * was done in order to afford an estimate of the difference, if any, caused by this practice.

1935-36.—In this year regular counts were taken weekly from the time of transplanting up to harvest. In addition to the bulk planting (Orchard Plots) at the normal time, a special early sowing (Border Plot) was made in June to see the result of sowing earlier in the monsoon. The sowing and planting out dates for the bulk crops were 23rd August and 3rd-4th October, respectively, and 22nd June and 25th August for the early crop. The latter was sown in two lots, the first in the open in the usual way and the second inside an insect-proof cage. Seedlings from both lots were planted out in the Border plot on the same day. The results are set forth in Tables II and III.

---

* Rogueing = removal of diseased plants which are then replaced with healthy ones.

TABLE II

Numbers and percentages of leaf-curl plants in special early sowing of H 142 tobacco in 1935-36

Border plot	Total No. of plants in field	Number of leaf-curl plant													
		August	September				October					November			Total
	Weekly counts →	5	1	2	3	4	1	2	3	4	5	1	2	3	
Planting from open nursery . .	150	1	1	16	7	3	19	4	17	35	2	1	0	1	107
		0·7	*0·7*	*10·8*	*5·3*	*2·4*	*15·6*	*3·9*	*17·2*	*42·7*	*4·3*	*2·2*	*0·0*	*2·3*	*71·3*
Planting from nursery in cage . .	150	4	8	21	13	7	10	1	22	16	1	5	0	0	108
		2·7	*5·5*	*15·2*	*11·1*	*6·7*	*10·3*	*1·1*	*25·6*	*25·0*	*2·1*	*10·6*	*0·0*	*0·0*	*72·0*
Average	300	5	9	37	20	10	29	5	39	51	3	6	0	1	215
		1·7	*3·1*	*12·9*	*8·0*	*4·4*	*13·2*	*2·6*	*21·1*	*34·9*	*3·2*	*6·5*	*0·0*	*1·2*	*71·7*

No rogueing was done. Figures in *Italics* are percentages.

TABLE

Numbers and percentages of leaf-curl plants in

Field	Variety	Total number of plants in field	Total number of plants including replacements	Number					
				October		November			
			Weekly counts →	4	5	1	2	3	4
Orchard 2-A .	H 142 *	4796	4885	36 *0·8*	13 *0·3*	40 *0·8*	15 *0·3*	9 *0·2*	21 *0·4*
Orchard 2-B and 2-C (part).	H 142 . .	6758	6758	20 *0·3*	87 *1·3*	61 *0·9*	65 *1·0*	52 *0·8*	73 *1·1*
Orchard 2-C .	Adcock † .	654	654	—	1 *0·2*	1 *0·2*	4 *0·6*	4 *0·6*	22 *3·4*
,, . .	Harrison's Special.	654	654	9 *1·4*	17 *2·6*	19 *3·0*	11 *1·8*	11 *1·8*	19 *3·2*
,, . .	Cash . .	654	654	13 *2·0*	19 *3·0*	12 *1·9*	20 *3·3*	12 *2·0*	8 *1·4*
,, . .	H 177 . .	763	763	—	6 *0·8*	15 *2·0*	25 *3·4*	23 *3·2*	23 *3·3*
Average . .		14279	14368	78 0·55	143 1·00	148 1·05	140 1·00	111 0·80	166 1·21

*Rogueing was carried out.

† Sown late (except for a small part which was sown at the same time as the others).

III

several varieties of tobacco in 1935-36 (*normal sowing*)

of leaf-curl plants															
December				January					February				March		Total
1	2	3	4	1	2	3	4	5	1	2	3	4	1	2	
32 *0·7*	25 *0·5*	10 *0·2*	17 *0·4*	3 *0·1*	1 *0·02*	3 *0·1*	0 *0·0*	0 *0·0*	3 *0·1*	1 *0·02*	1 *0·02*	0 *0·0*	1 *0·02*	61 *1·3*	292 ‡ *6·0*
31 *0·5*	47 *0·7*	23 *0·4*	19 *0·3*	13 *0·2*	1 *0·02*	10 *0·2*	10 *0·2*	0 *0·0*	2 *0·03*	7 *0·1*	4 *0·1*	2 *0·03*	0 *0·0*	12 *0·2*	539 *8·0*
12 *1·9*	12 *2·0*	2 *0·3*	6 *1·0*	1 *0·2*	0 *0·0*	2 *0·3*	2 *0·3*	0 *0·0*	1 *0·2*	0 *0·0*	1 *0·2*	0 *0·0*	0 *0·0*	4 *0·7*	75 *11·5*
2 *0·4*	9 *1·6*	5 *0·9*	4 *0·7*	0 *0·0*	0 *0·0*	4 *0·7*	3 *0·6*	0 *0·0*	1 *0·2*	0 *0·0*	1 *0·2*	1 *0·2*	2 *0·4*	22 *4·1*	140 *21·4*
12 *2·1*	8 *1·4*	6 *1·1*	5 *0·9*	2 *0·4*	0 *0·0*	1 *0·2*	4 *0·8*	0 *0·0*	3 *0·6*	2 *0·4*	27 *5·1*	5 *1·0*	5 *1·0*	62 *12·7*	226 *34·6*
11 *1·6*	20 *3·0*	4 *0·6*	5 *0·8*	0 *0·0*	0 *0·0*	2 *0·3*	3 *0·5*	0 *0·0*	1 *0·2*	2 *0·3*	2 *0·3*	0 *0·0*	0 *0·0*	3 *0·5*	145 *19·0*
100 0·74	121 0·90	50 0·37	56 0·42	19 0·14	2 0·02	22 0·17	22 *0·17*	0 *0·00*	11 *0·08*	12 *0·09*	36 *0·27*	8 *0·06*	8 *0·06*	164 *1·25*	1328 *11·5*

‡ The figure 292 includes the 89 leaf-curl plants discarded during 'rogueing'. and hence not strictly comparable.

The trend of the results may be comprehended readily by a reference to the graphs in Figs. 1 and 2.

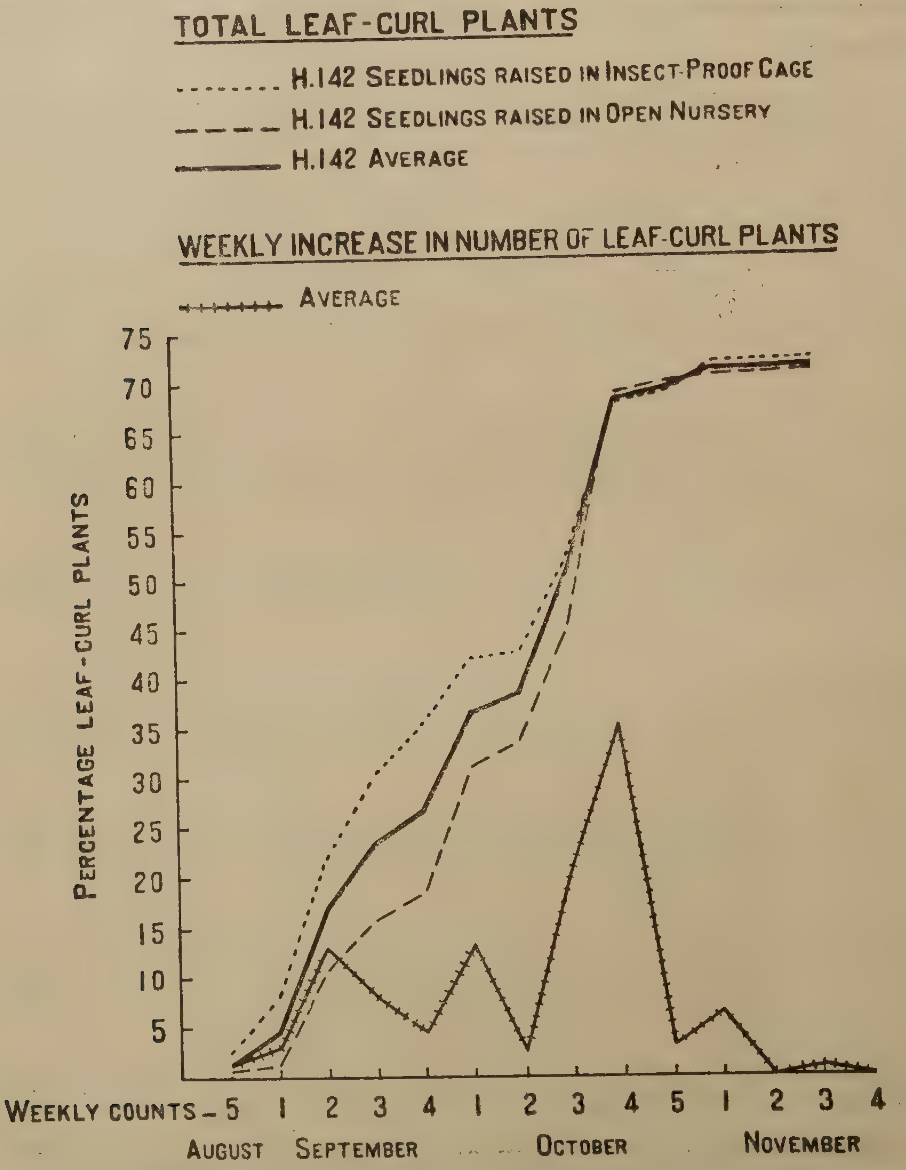


FIG. 1. Illustrating the amount and rate of increase of leaf-curl in successive weeks after transplanting in early-sown tobacco in 1935-36.

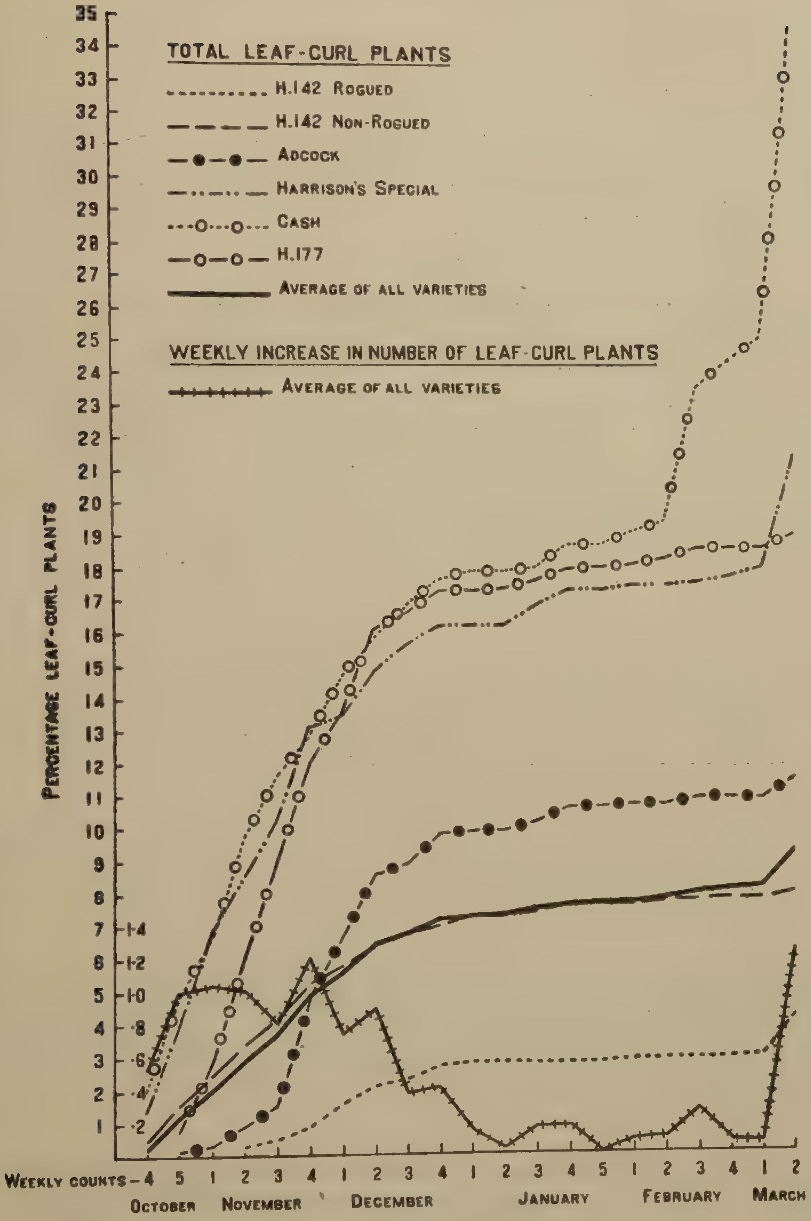


Fig. 2. Illustrating the amount and rate of leaf-curl in successive weeks after transplanting in normal-sawn tobacco in 1935-36.

Considering first the normal-sown tobacco (Fig. 2), it is obvious that the rate of spread is greatest, taking all the varieties together, at the end of October and in early November when the plants were planted out, and there are further large increases in the second half of November and early December. Thereafter there is a decline until the second week of January when no fresh infection at all was recorded, and this is followed by a small fluctuating amount of new infection. In March, however, a distinct rise is observed to take place, particularly in the case of the variety Cash.

The correlations between percentage weekly increase of leaf-curl plants and mean maximum temperature, mean minimum temperature, mean relative humidity and mean saturation deficiency (calculated from mean average temperature) for the twenty weeks over which counts were taken were, respectively, +0·459± 0·176, +0·542±0·158, —0·123±0·220, and +0·204±0·214. The correlations between percentage weekly increase of leaf-curl plants and the same meteorological factors for the week **before** the count were, respectively, +0·689±0·117, +0·633±0·143, —0·781±0·087 and +0·621±0·138. Temperature and humidity therefore evidently bear some relation to the rate of spread of the disease.

The very much higher percentage of disease in the early-sown plot (Fig. 1) is striking and presumably the conditions then prevailing were more favourable for the activity of the insect vector. In the case of this plot too there is a considerable variation in the amount of weekly infection.

The distribution of diseased plants in the Orchard 2-B plot (normal time sowing) is shown in Fig. 3 (also Plate XXII, fig. 2). The condition in this field was typical of that in other fields.

First appearance of disease symptoms.—In 1934-35, no disease was noted in the nurseries but at planting out time a very few isolated cases were observed. In 1935-36 only one plant was seen to be diseased in the nursery, out of a population of several thousand seedlings. In the case of the early-sown bed fourteen plants contracted disease.

As shown in Table II, seedlings grown under an insect-proof cage succumbed to disease as rapidly as those grown in open nurseries. This suggests that the major infection occurs after transplanting and not before. As, however, there is no definite knowledge regarding the incubation period (*i.e.* the interval that elapses between the introduction of the virus by the vector and the manifestation of externally apparent symptoms), this must be regarded as merely a surmise.

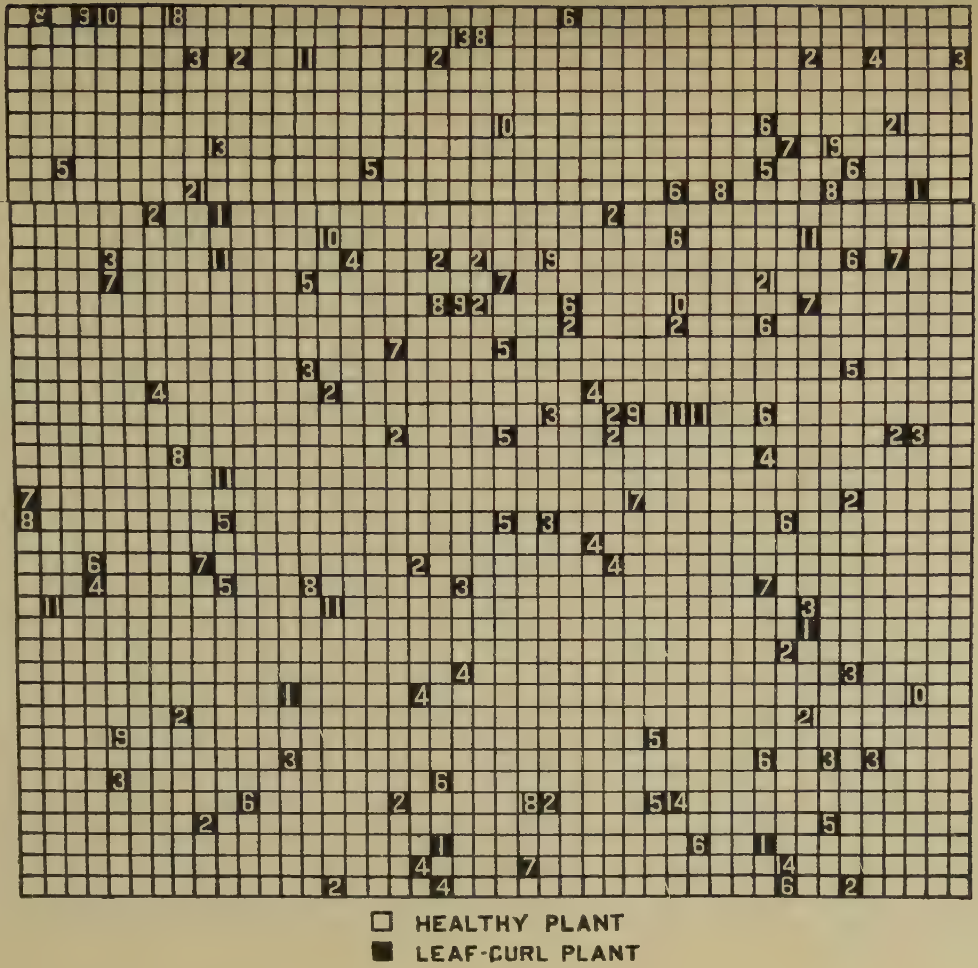


FIG. 3. Illustrating the distribution of leaf-curl plants in Orchard 2-B plot (normal time sowing) in 1935-36. (The number in each dark square indicates the week after transplanting in which leaf-curl symptoms were first noted)

LEAF-CURL PLANTS OF PUSA H 142 TOBACCO

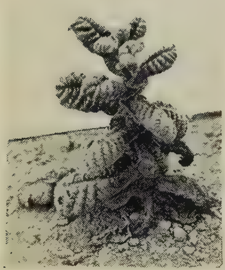


Fig. 1.
Young plant of *A* type.

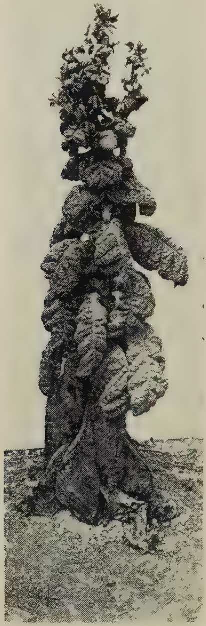


Fig. 2.
Full-grown plant of *A* type.

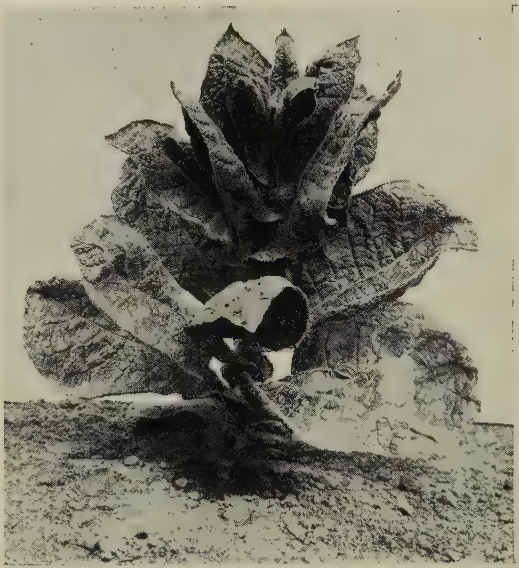


Fig. 3.
Plant of *B* type.

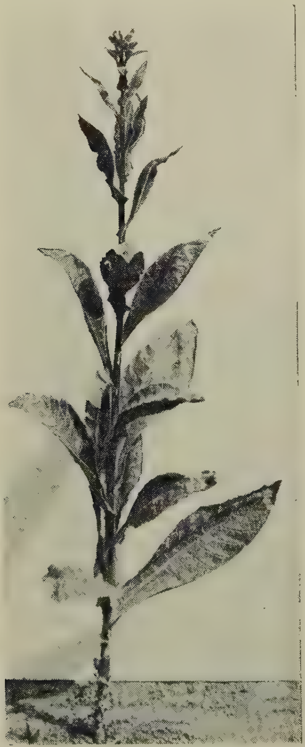


Fig. 4. Plant of *C* type.

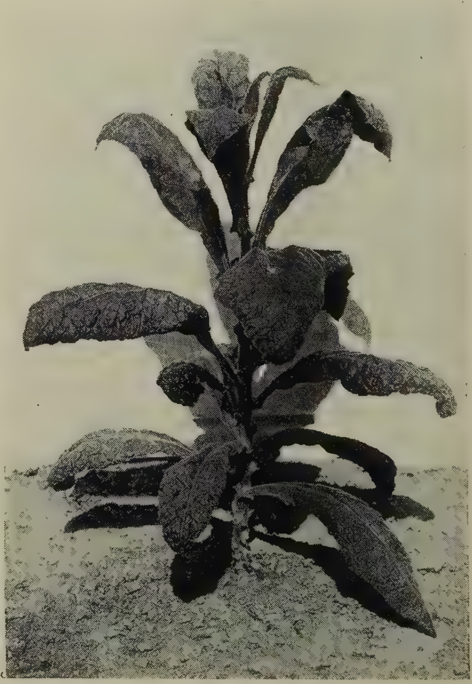


Fig. 5. Plant of *D* type.

(All photos are to the same scale except Fig. 3 which is on a larger scale)

V. TYPES OF LEAF-CURL

It soon became manifest that, as in Java, more than one kind of leaf-curl was present. Observations at Pusa over a period of two years, when over 10,000 diseased plants were available for study, indicate that five types of leaf-curl disease may be distinguished. These retain their characteristic symptoms with sufficient constancy to be recognised as distinct types : when typical plants of each kind are enclosed to exclude insects, there is no change from the symptoms of the type that is enclosed to those of any of the other types. It is our opinion that four of the five diseases described below are due to distinct viruses and that the fifth may be caused by a mixture of two or more of them.

The differentiation is perforce based on symptomatology as owing to the non-sap-transmissible nature of the diseases and the absence of definite knowledge of the vectors, the properties of the viruses concerned could not be determined. While it is known that specific viruses exist in nature with approximately the same constancy of manifestation as that exhibited by fungi and bacteria, there remains the possibility when discriminating such viruses by clinical expression alone, that a combination of two or more viruses may be mistaken for a single specific virus. But, as Johnson [1927] has pointed out, it is preferable in the absence of proof of this fact to treat each as distinct. There are certain features common to the leaf-curl diseases studied here which indicate that they belong to the same group. These features are :—

(1) A reduction in size of leaves and plant. The extent of the reduction varies, being greatest in the *A* type and least in the *C* type. The reduction in height is due to a shortening of internodes and not to any reduction in the number of nodes.

(2) Curling of portions or of the whole of the lamina. The degree of curling is again very variable. Sometimes curling may be absent except in a few of the youngest leaves.

(3) The presence of either vein-banding or of greening, or both, in some or all of the leaves. Vein-clearing appears to be a typical but transient phase in all the leaf-curl types and probably always precedes banding or greening. In the *C* type (and to some extent in the *D* type), vein-clearing is persistent.

(4) The inflorescence is abnormal, being generally much condensed. The flowers do not always open properly, the pigment is largely restricted to the veins of the corolla, and the veins of the calyx and ovary wall are thickened. The range of abnormality however is wide, extending from apparently normal flowers and capsules in mild types of *C* to a very greatly reduced type in *A* in which the buds drop without opening (Plate XXVII).

(5) All are transmissible by grafting but not by artificial inoculation. They are not carried in the seed; seeds obtained from bagged inflorescences of the several types were sown and all produced healthy seedlings. This is in agreement with the observations of Thung [1932] and Storey [1935].

The earliest stage at which leaf-curl has been identified is the 5-leaf stage (excluding cotyledons). It is not possible, however, to distinguish between the types in the very early stages when the usual characteristic picture is a curling of the younger leaves. Close examination at this stage will often reveal vein-clearing. Until greening sets in, however, the identification, even of leaf-curl in a broad sense, is not certain. In some cases seedlings that manifested marked curling later on developed into normal healthy plants. It is likely that these were cases of mistaken diagnosis rather than of recovery from the disease. At about six weeks after planting it is generally possible to discriminate between the different leaf-curl types.

As recommended by Johnson [1927] the virus in each case has been named separately from the disease. It is preferable however to indicate the affinities of the leaf-curl viruses by naming them as Tobacco Leaf-curl virus 1, etc. to distinguish them from the tobacco mosaic viruses which are termed as Tobacco Mosaic virus 1, etc. Some of the types of leaf-curl described are possibly the same as those found by Thung [1932]. Until this is proved, however, it is considered advisable to treat them as different entities.

Description of the leaf-curl diseases of tobacco

The symptoms are described as exhibited by the variety, Pusa H 142.

Tobacco leaf-curl A (*Tobacco leaf-curl virus 1*) [*Plate XXIII, figs. 1 and 2, Plate XXV, figs. 1 and 2, Plate XXVI, fig. 2*].—This is probably identical with the 'krulziekte' of Thung [1932]. The plant in Fig. 2 of Ludwig's paper [1913] also appears to be of this type.

Plant greatly dwarfed, height* about three feet only. Stem slightly zigzag, internodes very much reduced. Leaves much reduced in size except sometimes the lowermost ones (auricles however are not reduced to the same extent), thickened and curled downward uniformly at the margin, markedly rugose and brittle. The leaves generally retain a symmetrical appearance, are never rolled, and all except the topmost ones tend to droop. Foliage colour very dark green. Secondary veins tortuous. Transient vein-clearing probably present at an early stage. Secondary and smaller veins show much thickening and greening here and there. Enations numerous and of three kinds, *viz.* (*a*) frill-like (*b*) sessile and cup-like,

---

*The average height of healthy plants was about ten feet.

HEALTHY AND LEAF-CURL PLANTS OF PUSA H 142 TOBACCO

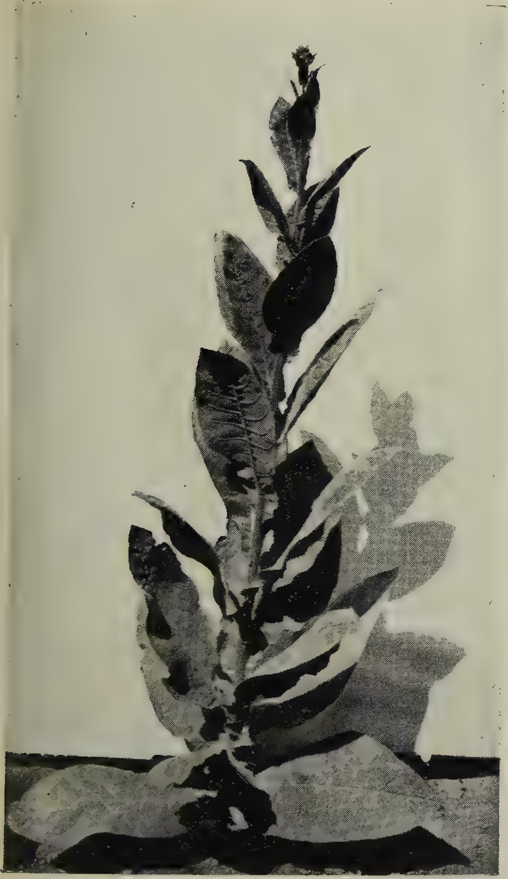


FIG. 1. Healthy plant.

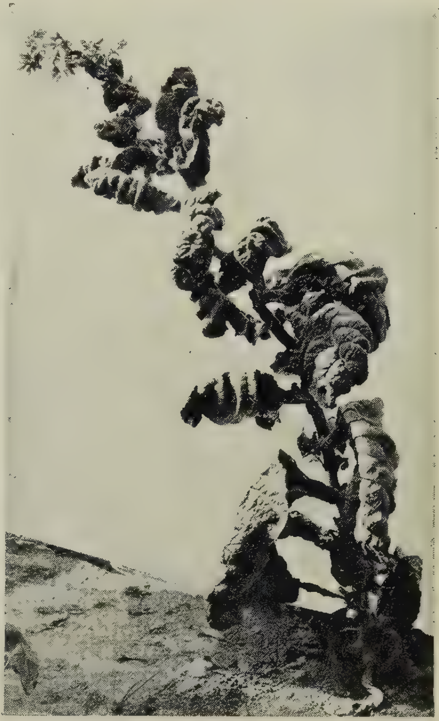


FIG. 2.

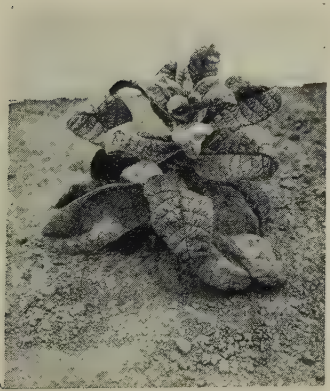


FIG. 3.

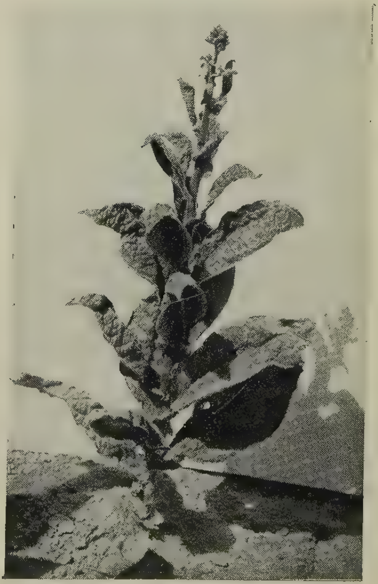


FIG. 4.

FIGS. 2, 3, and 4. Plants of *X* type showing range of variability.
(The plant shown in Fig. 2 is almost exactly intermediate between *A* and *B* types)
(Photos are to the same scale as those in Plate XXIII).

PUSA H 142 TOBACCO

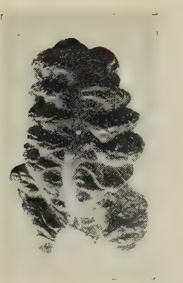


Fig. 1

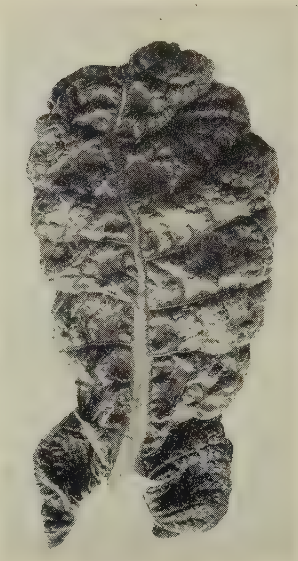


Fig. 2

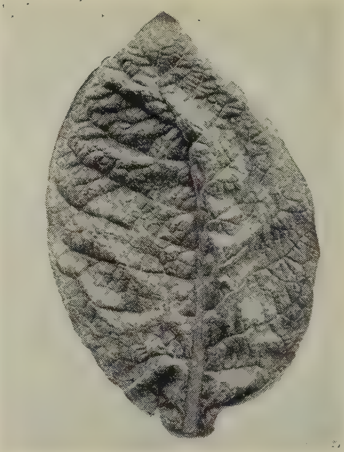


Fig. 4

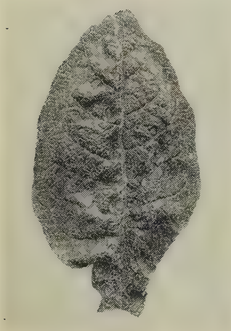


Fig. 3

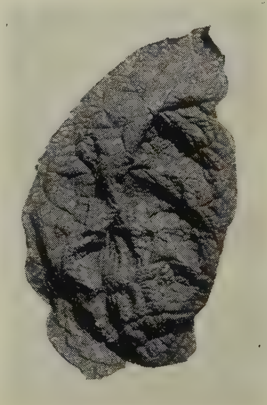


Fig. 7.

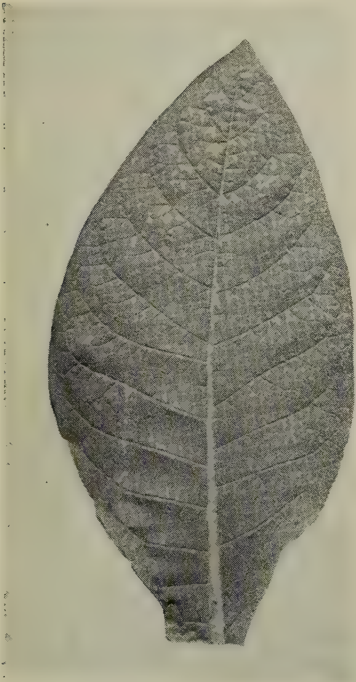


Fig. 8

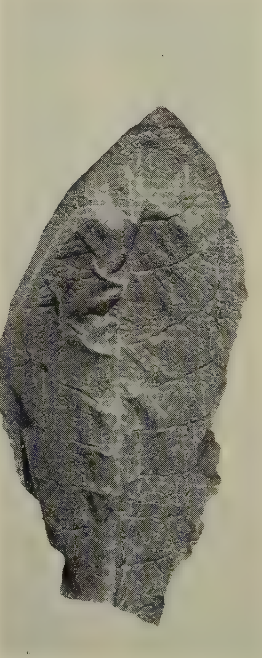


Fig. 5

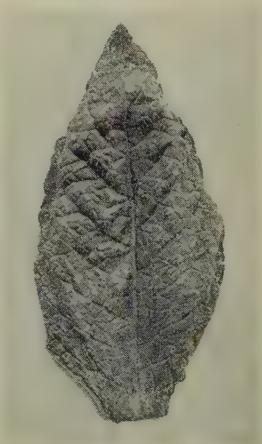


Fig. 6

FIGS. 1, 2. Young and old leaves from *A* type plants.

FIGS. 3, 4. Young and old leaves from *B* type plants.

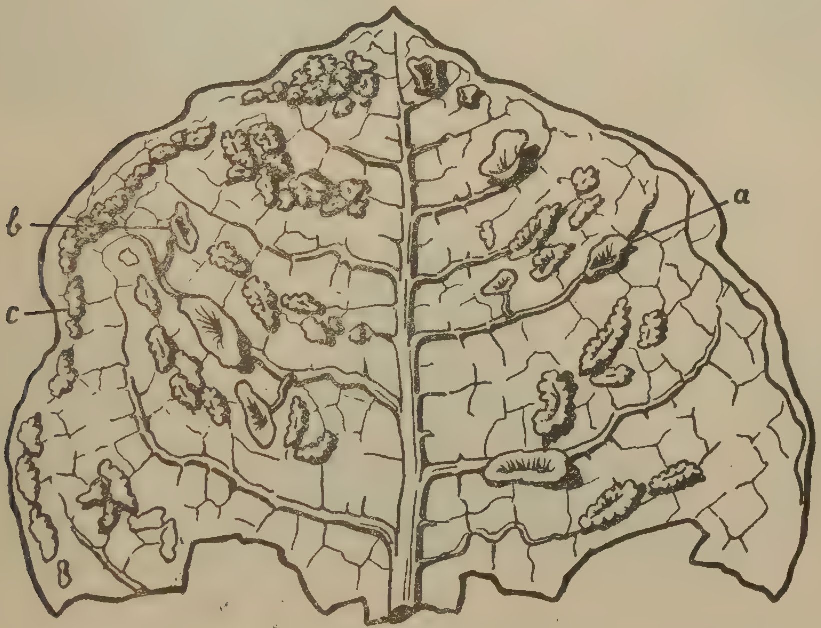
FIG. 5. Leaf from *C* type plant. Note 'broken rib' effect.

FIG. 6. Leaf from *D* type plant. The marginal 'gathering' characteristic of this type is however better seen in Plate XXIII, fig. 5.

FIG. 7. Leaf from *X* type plant.

FIG. 8. Leaf from healthy plant.

and (*c*) stalked and cup-like (Fig. 4). Greening and enations are mainly restricted to the area midway between each adjacent pair of secondary veins. Inflorescence very greatly reduced. Generally the buds drop without opening. Flowers when produced are small, opening imperfectly, almost colourless, the small quantity of pigment (which is brownish instead of clear pink as in the healthy flower) being restricted to the veins of the corolla ; veins of calyx and ovary wall much thickened. Capsules very rarely produced, small, containing ew and abnormal-looking seeds.



a = sessile, cup-like enation
b = stalked, cup-like enation
c = frilled enation

Fig. 4. Illustrating kinds of enations.

Tobacco leaf-curl B (*Tobacco leaf-curl virus 2*) [*Plate XXIII, fig. 3, Plate XXV, figs. 3 and 4, Plate XXVI, fig. 3*].—Plant greatly dwarfed, height about two feet only. Stem straight, internodes reduced. Leaves somewhat reduced in size, not thickened, curled slightly and uniformly at the margin, upper leaves sometimes rolled ; surface much wrinkled, sometimes even mildly rugose, not brittle. Leaves have a rounded and symmetrical appearance and do not droop ; they may however be ruffled. Foliage colour pale, yellowish green. Secondary veins tortuous but not to the extent shown by the veins in *C*. Vein-clearing transient.

There is thickening and greening of the secondary and smaller veins to form a more or less complete net. Enations characteristically absent but a few of all the kinds described for *A* may be rarely present; if so, they are usually small in size. Inflorescence reduced and condensed. Flowers do not open properly and the pigment is mainly restricted to the veins. Calyx veins much thickened. Capsules numerous; capsule wall not smooth. Sometimes leaf-curl plants are found which, while agreeing with the above description in other respects, possess darker leaves (normal green colour) and grow to a larger size than typical plants of *B*.

Tobacco leaf-curl C (*Tobacco leaf-curl virus 3*) [*Plate XXIII, fig. 4, Plate XXV, fig. 5, Plate XXVI, figs. 4 and 5*].—Either this or the next may correspond to the 'hellige kroepoek' of Thung [1932]. His description however is not sufficiently complete to enable this point to be settled.

Plant slightly or not at all reduced in stature, height* about eight and a half feet. Stem straight, internodes little or not reduced. Leaves little or not reduced in size, not thickened, slightly curled with a 'broken rib' effect due to marked waviness of the secondary veins. This is more pronounced on one side of the leaf than the other and gives an asymmetrical appearance to the leaf. Upper leaves sometimes rolled. Surface may be normal or wrinkled. Not brittle. Foliage colour normal green. Intense vein-clearing which extends to the smallest veins is present, and persists in the youngest leaves. This is the most characteristic symptom. In older leaves, vein-clearing is succeeded by vein-banding. General greening or enations absent, but occasionally in older leaves a small portion of a tertiary vein may show a green 'stitch'. Inflorescence slightly or not at all reduced. Flowers usually normal but sometimes do not open properly, and calyx veins may be slightly thickened. Capsules normal.

Tobacco leaf-curl D (*Tobacco leaf-curl virus 4*) [*Plate XXIII, fig. 5, Plate XXV, fig. 6, Plate XXVI, figs. 6 and 7*].—Plant dwarfed, height* about seven and a half feet. Stem straight, internodes reduced. Leaves somewhat reduced in size, not thickened, very slightly and uniformly curled at the margin, presenting a symmetrical appearance. Upper leaves sometimes rolled. Leaves, especially lower ones, much wrinkled at the margins. Generally the veins between each pair of secondary veins present a 'gathered' appearance (*i.e.* as though a string has been drawn through them). Not brittle. Foliage colour normal green in early stages, tending to become yellowish green with advancing maturity. Secondary veins tortuous. Vein-clearing usually persistent in younger leaves but not so distinct as that in *C* and moreover it does not extend to the smallest veins. Older leaves usually show vein-banding. Greening and enations entirely absent. Even

---

*The average height of healthy plants was about ten feet.

PUSA H 142 TOBACCO

(Portions of leaves photographed against the light)

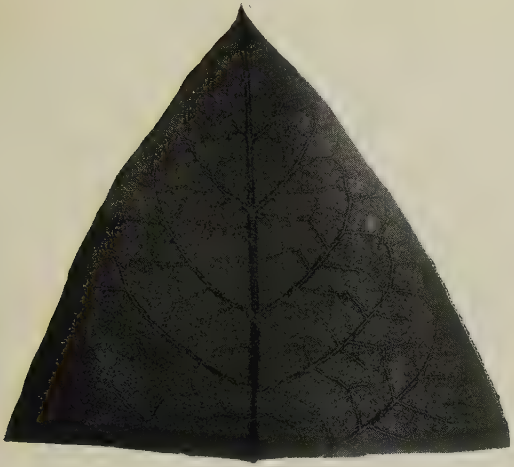


FIG. 1

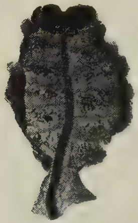


FIG. 2

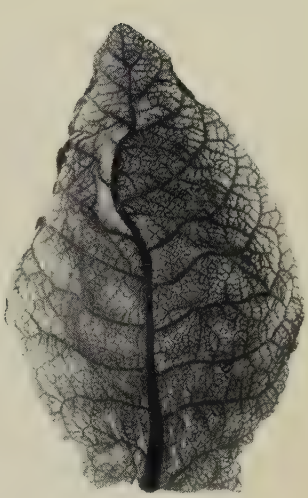


FIG. 3

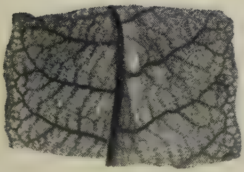


FIG. 4

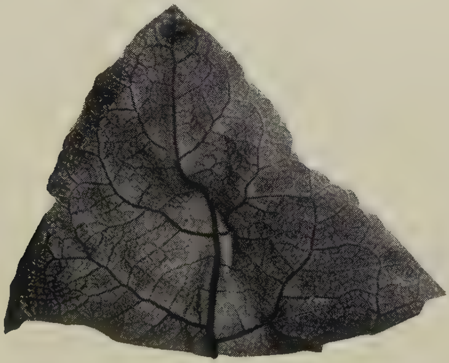


FIG. 5

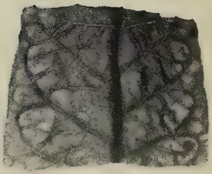


FIG. 6

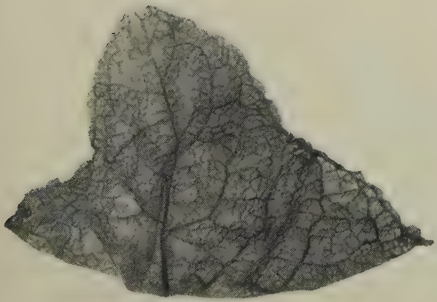


FIG. 7

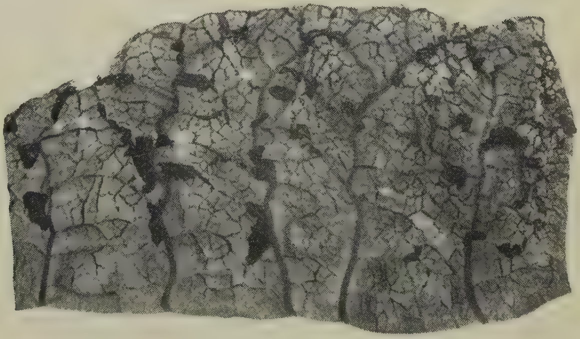


FIG. 8

FIG. 1. Normal.
FIG. 2. *A* type. Note thickening of veins, and presence of enations.
FIG. 3. *B* type. Note net-work of vein-thickening.
FIG. 4. *C* type. Young leaf showing vein-clearing.
FIG. 5. *C* type. Old leaf showing vein-banding.
Fig. 6. *D* type. Young leaf showing vein-clearing. Note difference in kind of vein-clearing from that found in *C*.
FIG. 7. *D* type. Old leaf showing vein-banding.
FIG. 8. *X* type. Note thickening of veins and numerous enations.

PUSA H 142 TOBACCO

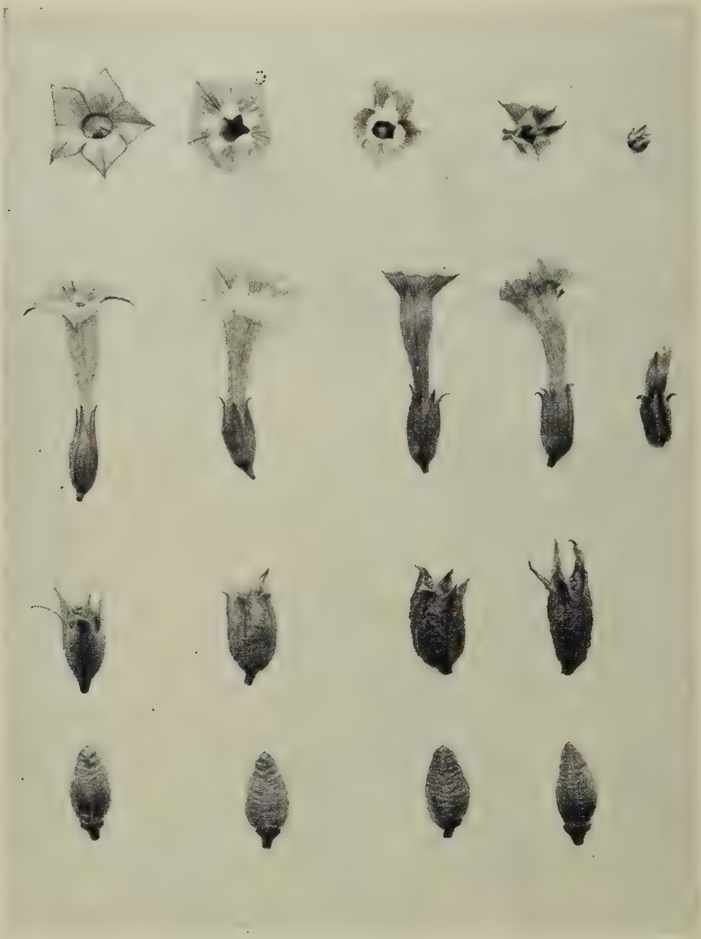


Fig. 1. Flowers and capsules from healthy (left) and leaf-curl plants. The ones on the extreme right are typical of *A* type plants. Note thickening of calyx veins in diseased flowers.

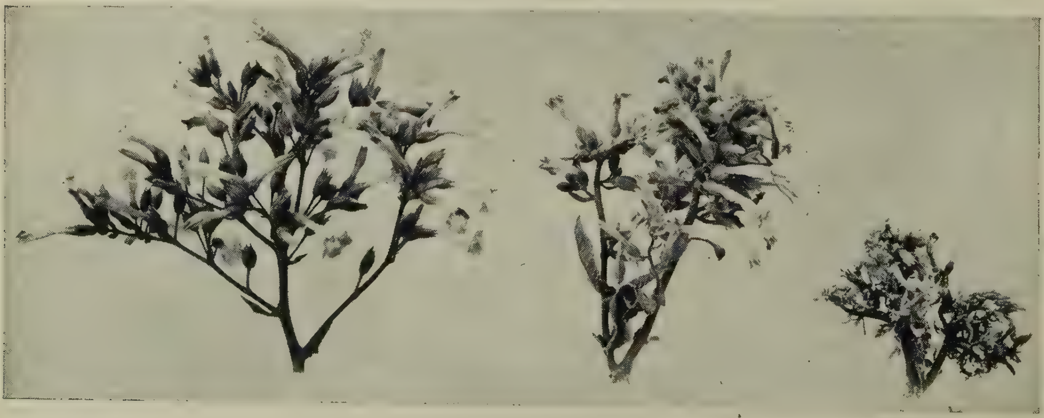


Fig. 2. Left, inflorescence from healthy plant ; middle, inflorescence from diseased plant ; right, inflorescence from *A* type plant. It is not possible to distinguish between inflorescences of *B*, *C*, *D*, and *X* types ; *C* is usually the least distorted.

green stitches such as occur sometimes in *C* are not found. Inflorescence reduced and condensed. Flowers do not open properly and the pigment is mainly restricted to the veins. Calyx veins much thickened. Capsules numerous, capsule wall not smooth.

Tobacco leaf-curl X [*Plate XXIV, figs. 2, 3 and 4, Plate XXV, fig. 7, Plate XXVI, fig. 8*].—This is considered to be caused by mixture of two or more of the viruses just described, in different combinations. The range of symptoms is wider and more variable than in any other of the foregoing. Attempts to produce this type synthetically by grafting pairs of leaf-curl plants of other types together are described in the next section. Leaf-curl *X* may correspond to Thung's 'gewone kroepoek'. The illustrations in Storey's paper [1935] also suggest this type.

Plants slightly to greatly reduced. Height very variable. Stem straight, zigzag or spirally twisted. Leaves slightly or much reduced in size, occasionally thickened, curled to varying degrees, sometimes the whole margin is involved, at other times only a part. Leaves sometimes rolled. Surface wrinkled, puckered or rugose. Leaves either symmetrical or asymmetrical, sometimes much twisted, sometimes, but not always, brittle. Foliage colour pale green, dark green or normal green. Secondary veins usually very tortuous. Vein-clearing usually transient, rarely persistent. Greening and enations of all kinds usually present. The latter are sometimes extremely large. Inflorescence flowers and capsules like that described for other types but very variable as regards degree of reduction, condensation, etc. Very rarely enations have been seen on the corolla.

Occasional plants were seen in the field which did not fall in any of the categories described. These plants were not investigated and it is possible that they were nonpathological cases. The commonest symptoms were stunting, chlorosis and slight rolling of the leaves there being no curling, vein-clearing, greening or enations.

The first four types ofl eaf-curl (*A*, *B*, *C*, and *D*) are readily separated whilst the symptoms of *X* are very variable and may approach those of any of the other types. It will be apparent that *A* is the most virulent type, with *B*, *D* and *C* following. *C* is the mildest and is not always easily distinguished from a healthy plant by a casual observer. Apart from the persistent vein-clearing, the other symptom most typical of this is the 'broken rib' effect of the lamina caused by the sudden upward and downward curvature of the secondary veins on their way to the margin ; this is usually more pronounced on one side of the leaf than on the other. The 'broken rib' appearance however is typical of some varieties, *e.g.* Pusa H 156, in the healthy state.

TABLE IV

Numbers and percentages of different types of leaf-curl plants in 1935-36

Variety	Leaf-curl *A*		Leaf-curl *B*		Leaf-curl *C*		Leaf-curl *D*		Leaf-curl *X*		Not identified*	
	Total	Per cent	Total	Per cent	Total	Per cent	Total	Per cent	Total	Per cent	Total	Per cent
EARLY SOWING												
H 142 (Orchard Border).	20	*9·0*	33	*14·9*	23	*10·4*	7	*3·1*	136	*61·3*	3	*1·3*
NORMAL SOWING												
H 142†	7	*2·4*	24	*8·2*	73	*25·0*	29	*9·9*	69	*23·6*	90	*30·8*
H 142	46	*8·5*	112	*20·8*	90	*16·7*	46	*8·5*	229	*42·5*	16	*3·0*
Adcock	6	*8·0*	19	*25·3*	17	*22·7*	11	*14·6*	21	*28·0*	1	*1·3*
Harrison's Special	12	*8·6*	14	*10·0*	29	*20·7*	11	*7·9*	74	*52·9*	0	*0·0*
Cash	19	*8·4*	24	*10·6*	92	*40·7*	25	*10·6*	65	*28·8*	1	*0·4*
H 177	8	*5·6*	45	*31·0*	15	*10·3*	36	*24·8*	40	*27·6*	1	*0·7*
Average	16·3	*6·9*	39·7	*16·8*	52·7	*22·3*	26·3	*11·2*	83·0	*35·1*	18·1	*7·7*

---

* This comprises (*a*) leaf-curl plants which were rogued before identification, (*b*) leaf-curl plants which developed mosaic also and were pulled out.

† Rogued. No rogueing was done in the others.

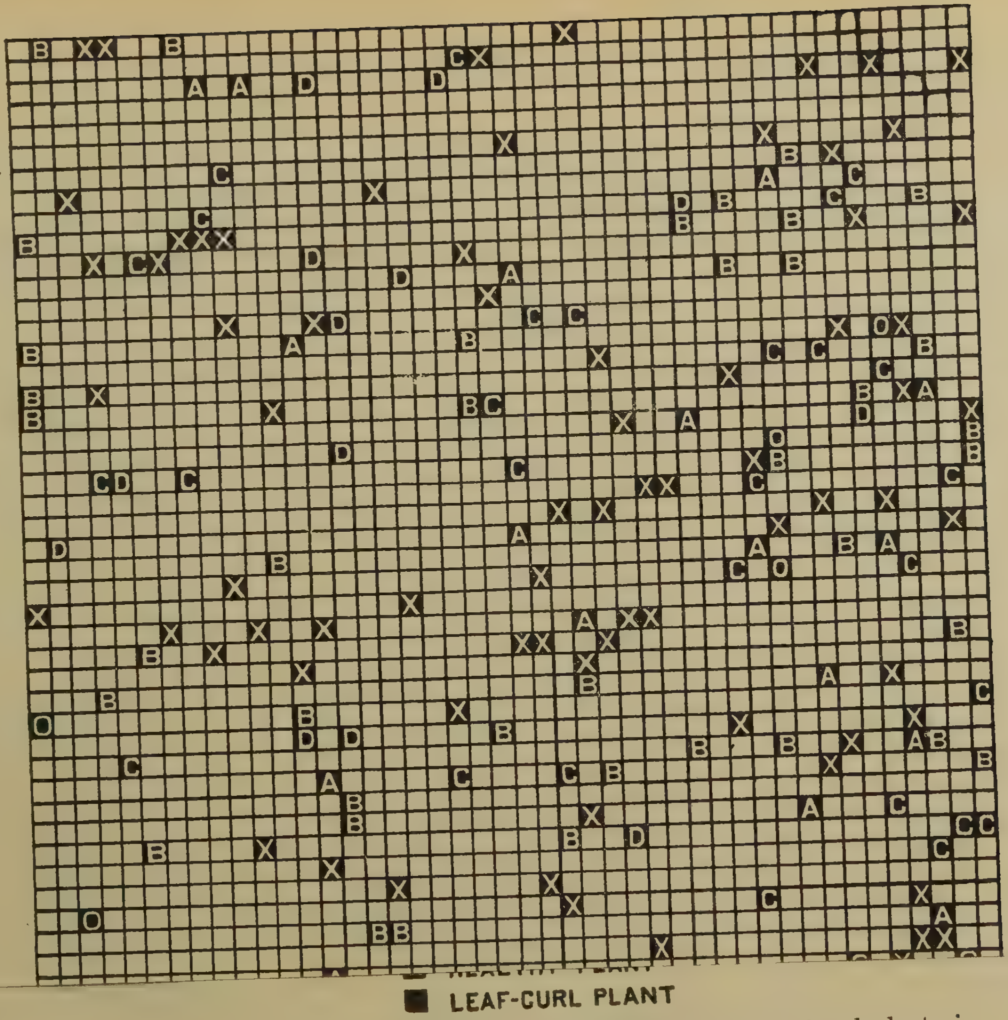


FIG. 5. - Illustrating the distribution of different types of leaf-curl plants in Orchard 2-B plot (normal time sowing) in 1935-36.

A=*A* type leaf-curl plant
B=*B* type leaf-curl plant
C=*C* type leaf-curl plant
D=*D* type leaf-curl plant
X=*X* type leaf-curl plant
O=Not definitely identified

The relative numerical importance of the leaf-curl types in 1935-36 can be seen from Table IV and Fig. 5. It will be noticed that the *X* type was the most common whilst *A* and *D* were more rare. *D* also appeared relatively late in the season.

Inoculation and grafting tests.—Sets of ten healthy plants each were inoculated with the juice of diseased plants of *A*, *B*, *C*, *D*, *X* and healthy (control) types, respectively. In no case was any apparent reaction produced. At the same time, for each type, three healthy and three diseased plants were grafted, making six grafts for each type. In every case the originally healthy component of the graft showed disease symptoms of the particular type with which it was grafted. When the healthy plant was scion and the diseased plant stock, the time generally taken for symptoms to appear was about three weeks. For the reciprocal where the symptoms manifest themselves only in the new growth (suckers) from the stock the time taken was longer. Six grafts of healthy grafted on healthy, as a control, remained disease-free.

Symptoms on differential hosts.—Shoots of healthy plants (raised under wire-mesh cages) of *N. Tabacum* var. Turkish, *N. rustica* var. Pusa T 18, *N. glutinosa*, *Solanum nigrum*, *Lycopersicum esculentum* and *Petunia* sp. (garden variety) were grafted on diseased plants of Pusa H 142 tobacco showing symptoms of *A*, *B*, *C*, *D* and *X* types of leaf-curl and also on healthy plants of it (control). To prevent any possibility of admixture of viruses while grafting , each disease type was handled on a different day and the grafting operations were performed on six successive days, the control being kept in about the middle of the series. In most cases only two grafts could be made. With the exception of the *X* type (which as already mentioned appears to be a mixture) the symptoms exhibited by each pair were remarkably uniform and indicated that in spite of the handicap of small numbers, some measure of reliance could be placed upon the results. The latter are presented in Table V.

TABLE V

Reactions of differential hosts when grafted on plants of H 142 tobacco suffering from A, B, C, D *and* X *types of leaf-curl*

Type of leaf-curl	*N. Tabacum* var. Turkish	*N. rustica*	*N. glutinosa*	*Solanum nigrum*	*Lycopersicum esculentum*	*Petunia* sp.
A	*	Pronounced curling, slight rugosity, net-work of greening (mostly burst), leathery texture	. .	Slight greening of veins on underside of leaf	. .	Unburst greening
B	Vein-banding with a few green 'stitches'	Slight twisting of leaves, net-work of greening (mostly unburst)	. .	Slight greening of veins on underside of leaf	. .	Unburst greening
C	Vein-clearing	Faint net-work of unburst greening	Intense vein-clearing	. .	Vein-clearing	Slight vein-clearing
D	†	No greening, but small dots like unburst greening present in lamina	Vein-clearing	. .	Vein-clearing	Distinct vein-clearing
X	Greening and enations	Slight curling. Net-work of greening (burst in a few or many places)	. .	. .	. .	Burst and unburst greening (one plant showed vein-clearing)

* Scion did not grow.

† Not attempted.

CHEWING TOBACCO, PUSA TYPE 63

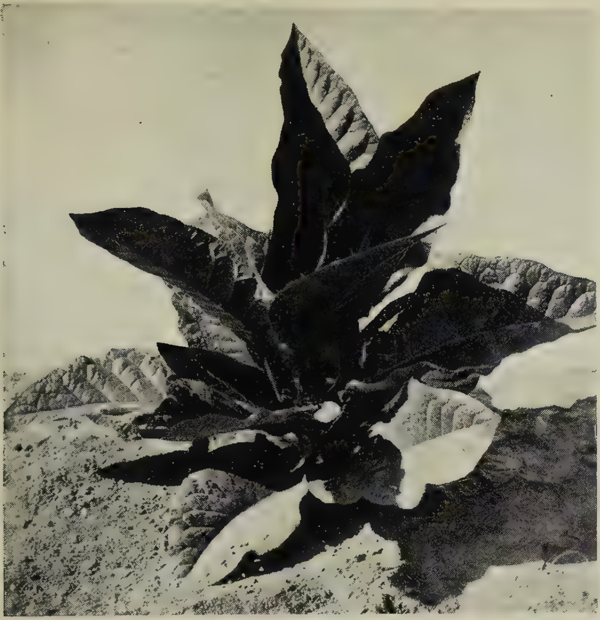


FIG. 1. Healthy plant.

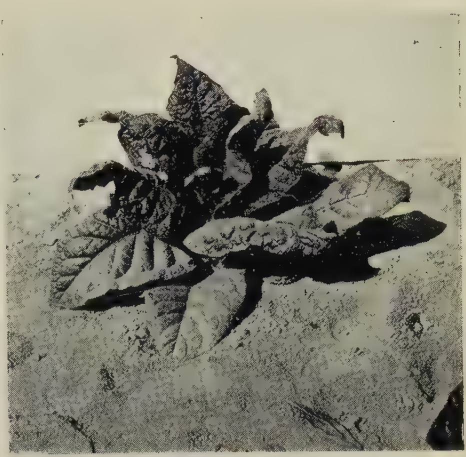


FIG. 2. Plant with Tobacco Leaf-curl virus 1 (*A* type leaf-curl).

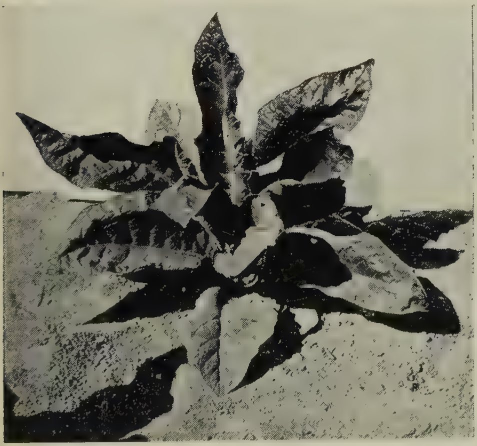


FIG. 3. Plant with Tobacco Leaf-curl virus 3 (*C* type leaf-curl).

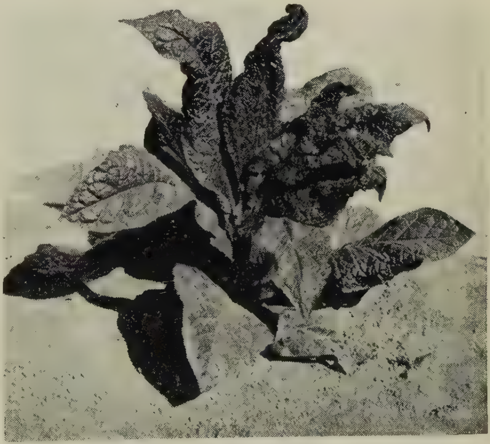


FIG. 4. Plant with Tobacco Leaf-curl virus 4 (*D* type leaf-curl).

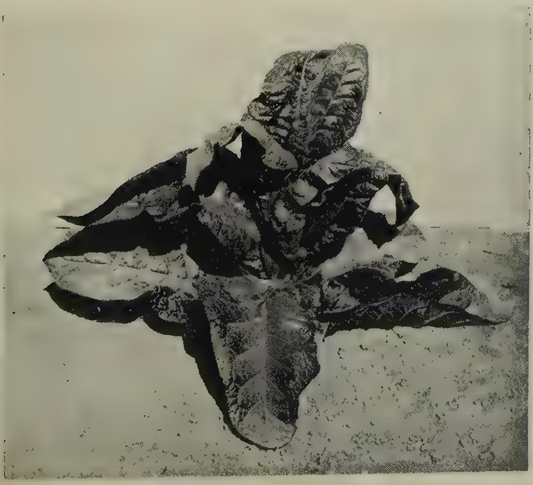


FIG. 5. Plant with *X* type curl.

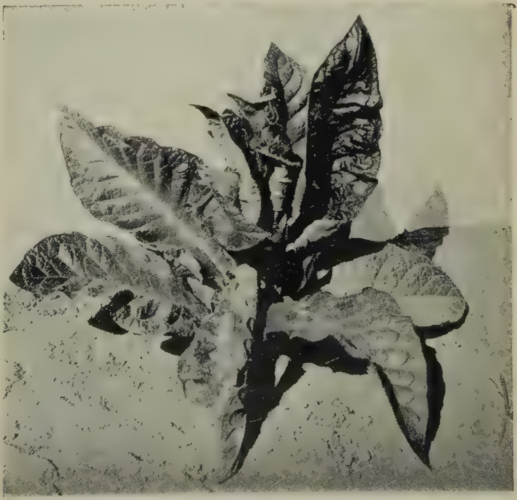


FIG. 6. Plant which showed rolling of leaves and slight vein-clearing. Not identified.

CHEWING TOBACCO, PUSA TYPE 63

(Upper view of leaves)

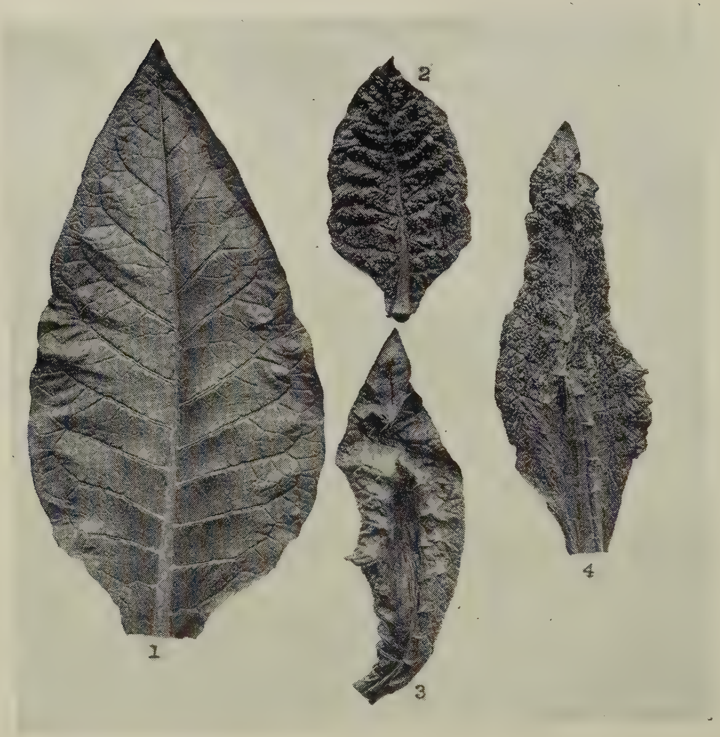


FIG. 1. Healthy. FIG. 2. *A* type. FIG. 3. *C* type. FIG. 4. *D* type.

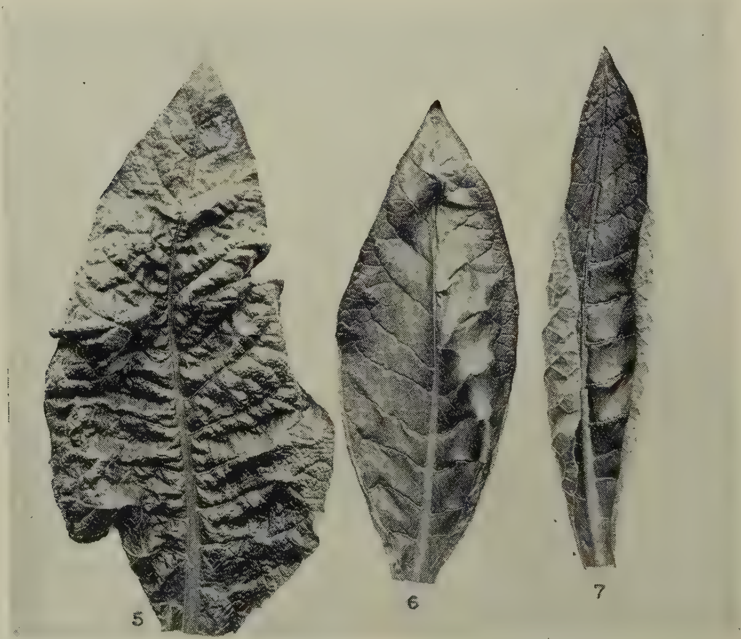


FIG. 5. *X* type. FIGS. 6 & 7. Unidentified type, characterised by rolling of leaves.

A and *B* can be easily separated from *C* and *D* by their symptoms on *N. glutinosa*, *S. nigrum* and *Petunia*. *A* can be separated from *B* and *C* from *D* by the symptoms on *N. rustica*. *X* is, of course, very variable.

Symptoms on different tobacco varieties

The symptoms as described for Pusa H 142 do not necessarily hold true for different varieties of tobacco. For the group of cigarette tobacco varieties (Harrison's Special, Adcock, Cash, etc.) however the divergence is not great and the leaf-curl types can be readily identified in them. This is not so, however, with the chewing tobacco group (Pusa T 28, T 58 and T 63) where the symptoms are distinctly different from those on Pusa H 142.

As the symptoms in Pusa T 28, T 58 and T 63 differ but slightly from each other only those on T 63 are described. These are :—

A [*Plate XXVIII, fig.* 2, *Plate XXIX, fig.* 2, *Plate XXX, fig.* 3].—Plant and leaves slightly reduced, leaves curled, much puckered and even rugose, leathery ; older leaves show yellowish blotches. Small to medium-sized enations of all kinds present in large numbers and also greening (' here and there ' type).

When healthy scions of Pusa H 142 were grafted on diseased plants of this type, the former developed typical symptoms of leaf-curl disease, type *A*.

C [*Plate XXVIII, fig.* 3, *Plate XXIX, fig.* 3, *Plate XXX, fig.* 4].—Plants and leaves not reduced. Leaves show ' broken rib ' effect, and are ruffled and twisted. Extensive vein-clearing present, followed by banding in older leaves. A few green ' stitches ' may occur. When healthy scions of Pusa H 142 were grafted on diseased plants of this type, the former developed typical symptoms of leaf-curl disease, type *C*.

D [*Plate XXVIII, fig.* 4, *Plate XXIX, fig.* 4, *Plate XXX, fig.* 5].—Plant and leaves not reduced. Midrib and secondary veins tortuous. Upper leaves much curled ; ' gathered ' effect at margins suggests a virulent form of *D* type leaf-curl. Leaf surface much wrinkled except near midrib. Extensive net-work of vein-banding present but no greening or enations. When healthy scions of Pusa H 142 were grafted on diseased plants of this type, the former developed typical symptoms of leaf-curl disease, type *D*.

X [*Plate XXVIII, fig.* 5, *Plate XXIX, fig.* 5, *Plate XXX, fig.* 2].—Plant and leaves not reduced. Leaves much ruffled and slightly curled, secondary veins tortuous (sometimes midrib also). Middle and upper leaves wrinkled. Complete net-work of vein-banding and greening present but no enations. When healthy scions of Pusa H 142 were grafted on diseased plants of this type, the former developed symptoms typical of a particular type of *X*. The wide variability exhibited by *X* on Pusa H 142 is here absenit.

Unidentified type [*Plate XXVIII, fig. 6, Plate XXIX, figs. 6, 7*].—The only symptoms are rolling of the upper leaves, sometimes accompanied by slight vein-banding. Grafting with healthy Pusa H 142 produced only slight vein-clearing in upper leaves of the latter. It is not yet possible to say whether this is a distinct virus and whether it corresponds to any of those found in Pusa H 142.

It will be noted that the *B* type was not recovered from chewing tobacco. Further it will be observed that whereas *C* and *D* are rather alike on Pusa H 142, in Pusa T 63 the difference between them is very clear.

On *N. rustica* the symptoms of leaf-curl viruses are rather similar, consisting of curling and ruffling of the leaves accompanied by an incomplete net of 'unburst greening' (see Section III, last paragraph) which however 'bursts' in several places. No vein-clearing or vein-banding has been noticed, and there is little or no reduction in size of leaves and plant (Plate XXXI, figs. 1-4). Of two diseased plants of *N. rustica* which were grafted with Pusa H 142 both transmitted *X* type of leaf-curl, the symptoms in one case approaching those of *A* and in the other case those of *C*.

It is obvious that much remains to be done to clear up the relations of the virus diseases described. It is likely that a study of the properties of the viruses concerned and the entomological investigations being conducted by the Imperial Entomologist may throw some light on this. Fully effective control measures can probably be devised only after the elucidation is complete.

VI. ATTEMPTS TO PRODUCE LEAF-CURL VIRUS *X* SYNTHETICALLY

That the '*X*' type of leaf-curl may be the result of a mixture of two or more leaf-curl viruses has already been indicated in a previous section. In order to demonstrate its composite nature attempts were made to produce it synthetically. The fact that all these viruses are not juice-transmissible entailed certain difficulties and all the tests had perforce to be made by grafting. The grafts were made according to the following scheme :—

	Scion	Stock
(1)	'*A*' leaf-curl virus	'*B*' leaf-curl virus
(2)	'*B*' leaf-curl virus	'*A*' leaf-curl virus
(3)	'*A*' leaf-curl virus	'*C*' leaf-curl virus
(4)	'*C*' leaf-curl virus	'*A*' leaf-curl virus
(5)	'*B*' leaf-curl virus	'*C*' leaf-curl virus
(6)	'*C*' leaf-curl virus	'*B*' leaf-curl virus

('*D*' was not used in this series as enough plants were not available at the time grafting was done).

CHEWING TOBACCO, PUSA TYPE 63

(Leaves photographed against the light)

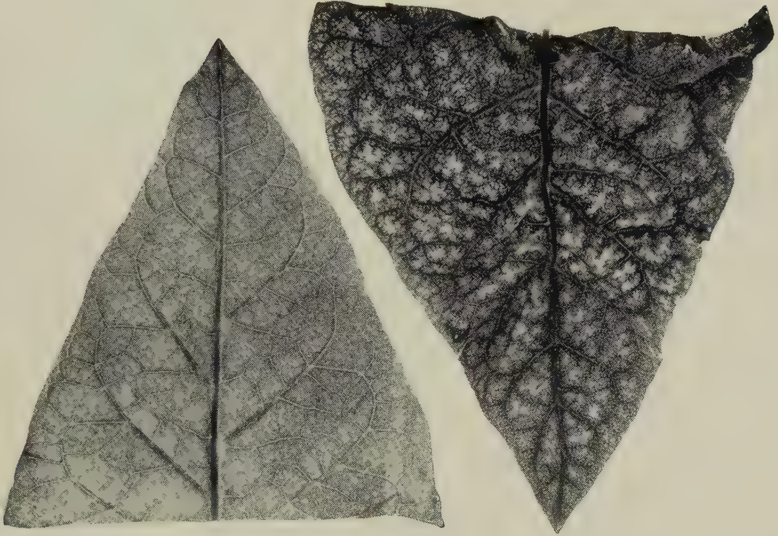


FIG. 1. Healthy.

FIG. 2. *X* type. Note thickened net of veins.

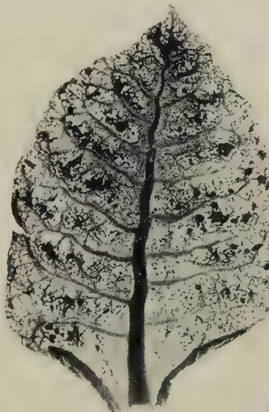


FIG. 3. *A* type.

Note enations and vein-thickening.

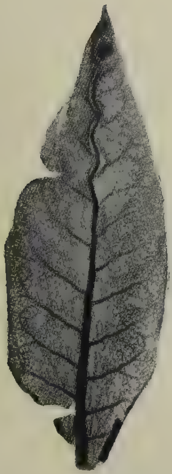


FIG. 4. *C* type.
Note clearing and banding of veins.

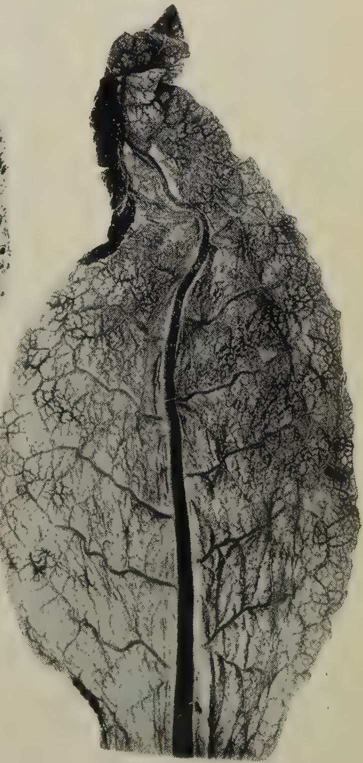
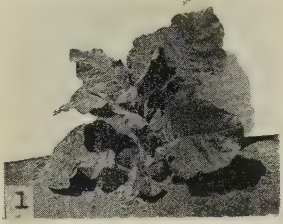
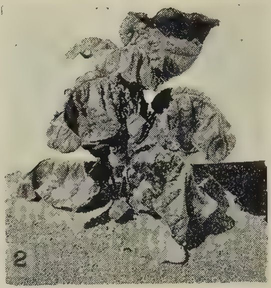
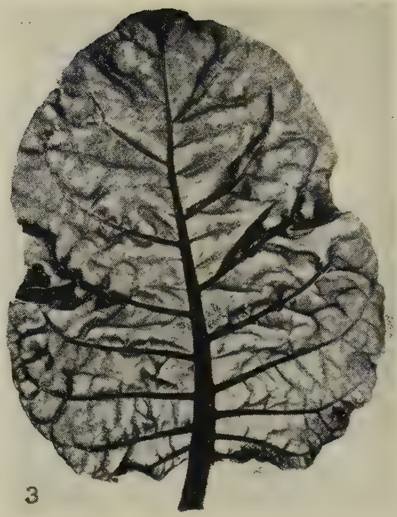


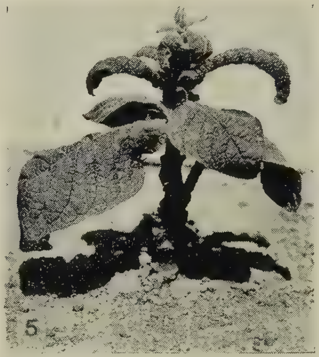
FIG. 5.

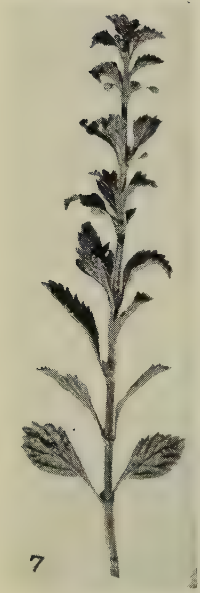
FIG. 5. *D* type. Note 'gathering' of leaf at the margins and slight vein-banding.

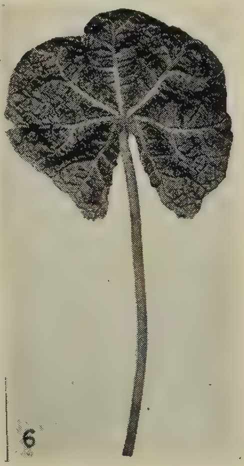












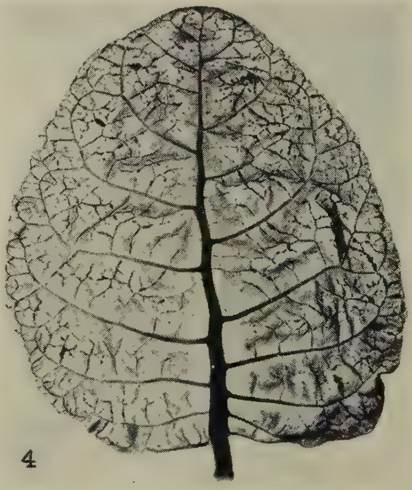


Fig. 1. Healthy plant of *N. rustica,* Pusa Type 18.

Fig. 2. Leaf-curl plant of *N. rustica,* Pusa Type 18.

Fig. 3, 4. Healthy and diseased leaves of *N. rustica,* Pusa Type 18, photographed against the light. Note thickening of veins in the latter.

Fig. 5. *X* type leaf-curl produced by grafting *A* and *B* types together.

Fig. 6. Leaf of hollyhock showing thickening of the veins on the underside.

Fig. 7. Leaf of *Scoparia dulcis* showing greening and outgrowths of veins.

All the grafts were made taking the usual aseptic precautions and there were six, two and four pairs respectively of each combination. The appearance of the grafts after about eight weeks indicated the manner in which the different combinations reacted in producing a resultant symptom-picture. The results are summarised below :

(1) It appeared as though the characters of *A* and *B* had united in the new growth which the scion put forth, the characteristics of *A* however predominating. The leaves were thick and rugose with numerous enations of several kinds but they were not brittle or thick to the extent typical of *A* leaf-curl virus. The suckers from the stock showed almost purely *A* characters indicating the downward path of the virus.

(2) This was like (1) but predominance of *A* was even more complete (Plate XXXI, fig. 5).

(3) Scion remained like *A* but the colour was rather lighter and the leaves were not so thick and brittle as is typical of *A*. The appearance suggested a slightly 'diluted' form of *A*.

The suckers from the stock presented an appearance intermediate between *A* and *C* such as is often found in *X* type plants in the field.

(4) Like (3), the characters of *A* predominating.

(5) Scion remained in general like *B* but lower leaves showed a distinct rolling. The net-work of greening developed slowly and rather imperfectly. Vein-clearing persisted in the youngest leaves. Suckers were inadvertently removed before notes could be taken.

(6) Scion remained as *C* but the number of green 'stitches' was slightly increased and the leaves were rather more wrinkled than is typical for *C*.

It will be apparent that *B* and *C* when grafted together reacted rather weakly to each other and each component of the graft tended to retain its own characters. Leaf-curl *A* on the other hand tended to dominate over the others with which it was grafted. It is interesting to recall here the instance given by Salaman [1933] of a virus being able, once it is established in a plant, to afford protection against another similar virus or virus strain. Kunkel [1936] also discusses the phenomenon and is of opinion that such immunity reactions indicate a close relationship between viruses.

As a sequel to the above tests it was decided to study the effect of grafting portions of plants of two distinct leaf-curl viruses on a healthy plant at the same time. For this purpose healthy plants of Pusa H 142 (from the field) which had been bagged and under observation for a month, were topped. This promoted the production of suckers of which the uppermost three were retained. The lowest of these was grafted with a scion of what appeared to be the more virulent virus and the one above it with a scion of the less virulent virus. The top-most sucker was allowed to remain as an 'indicator'. The following combinations, in duplicate, were made :

(1) *A* and *C* (3) *B* and *D*
(2) *B* and *C* (4) *C* and *D*

Symptoms appeared in the indicators within thirteen to twenty-six days after grafting and the results were as follows :

(1) Indicator showed symptoms (vein-banding, greening and pin-head enations) exactly like those of a type of *X* commonly found naturally in the field.

(2) Indicator showed symptoms typical of *X*. Differed however from the foregoing in that there was less rugosity and no enations though there was an extensive net-work of greening. Also slight vein-clearing persisted in the youngest leaves.

(3) Like (2) but leaves rather more curled and rugose. In addition to the net-work of greening some frill-like enations were present.

(4) Leaves of the indicator were 'gathered' at the margin like *D*. Middle leaves showed the *D* type of vein-clearing and the youngest ones the *C* type of the same. Older leaves showed slight vein-banding but there was no greening at all and no enations.

With the exception of the *C-D* combination which was different, the other three combinations produced in the indicators symptoms corresponding to those manifested by various *X* type leaf-curl plants under normal field conditions. The results indicate therefore that the variety of form found in the *X* type is probably largely to be attributed to more than one virus being present in the plant in more than one combination. The time at which the viruses enter the plant would affect the symptom-expression and also the point at which infection takes place if Kunkel's conclusions regarding certain virus diseases of the peach [Kunkel, 1936] can be applied to other viruses. It is only after the vectors responsible for the transmission of the various leaf-curl viruses have been definitely discovered that more information with regard to the components of the *X*-virus can be obtained. The grafting tests demonstrate, however, that the assumption that the *X* type of leaf-curl is a mixture of other leaf-curl viruses is to a large extent true.

VII. ALTERNATE HOSTS AND THE PROBLEM OF CONTROL

Possible measures of control are :—

1. Removal of ratooning and self-sown tobacco plants, and of alternate hosts.
2. Rogueing of fields.
3. Protection from insect vectors.
4. Breeding resistant varieties.

These will be discussed *seriatim*.

1. Tobacco being an annual, the question of the mode of perpetuation of the leaf-curl viruses immediately arises. If the cycle can be broken, then the disease can be controlled. The thorough cleaning of tobacco land after the crop is harvested and the removal of weeds harbouring the virus has been strongly advocated by Storey [1932], Hornby [1932], Mossop [1932], Smee [1932], Thung [1934], etc. In fact in South Africa a Tobacco Pest Suppression Act provides that " By the first day of August in any year the owner of any land shall have caused to be uprooted and destroyed all growing tobacco plants, including living stalks and roots, and all plants declared to be alternate hosts, which have been growing on his land " (*Rhod. Agric. J.* **32**, 776-79 : 1935).

Transmission of leaf-curl to tobacco has been secured by various workers from the following plants :

Vernonia iodocalyx [Storey, 1935], *V. cinerea*, *Ageratum conyzoides*, and *Synedrella nodiflora* [Thung, 1934]. Also suspected are *Cucumis sativus*, *Manihot utilissima* [Thung, 1934], *Sida* [Storey, 1935], etc. Of these *Vernonia cinerea*, *Ageratum conyzoides* and *Sida rhombifolia* are common weeds in Pusa while *Sida humilis* occurs to a lesser extent. No greening or enations have been noted on them during the tobacco season, but soon after the break of the rains symptoms of ' curl ' (curling, puckering, greening, etc.) were frequently observed. A healthy plant of tobacco when grafted upon *Ageratum conyzoides* developed vein-clearing but unfortunately the graft died before other symptoms could be noted. Twisting and curling of leaves reminiscent of tobacco leaf-curl have been noticed at Pusa upon various cucurbits, *Mina lobata*, *Carica papaw*, *Datura stramonium*, *Physalis peruviana*, *Capsicum annuum*, etc., while greening or both greening and enations have been noted on *Zinnia elegans*, *Petunia* sp., *Althaea rosea* (hollyhock), *Hibiscus rosa-sinensis*, *Crotalaria juncea* and a common perennial weed, *Scoparia dulcis* (Plate XXXI, fig. 7). Pusa H 142 tobacco grafted on a diseased plant of *Datura* manifested symptoms like *C* type of leaf-curl.

N. plumbaginifolia is present as a weed all the year round but shows no symptoms : it may either be resistant or a carrier.

The group of alternate host plants is probably a large one and until all the members composing it are discovered, it will not be possible to control leaf-curl effectively by this method.

In Java only three species (*Ageratum conyzoides*, *Vernonia cinerea* and *Synedrella nodiflora*) appear to be important as alternate hosts and Thung [1934] states that control of leaf-curl has been secured by the timely eradication of these weeds up to a distance of fifty metres from the tobacco.

2. Removal of diseased plants and replacement with healthy ones is a routine followed by some cultivators. It is possible however to do this only up to a certain stage of growth as plants set out very late will never make enough growth to catch up with the early-planted ones.

In 1934-35 when the disease was severe two fields of Pusa H 142 tobacco of 0·65 acre each were rogued and resulted in a decrease of diseased plants of about twenty per cent as compared to the non-rogued fields, in the final counts. In 1935-36 (a year of mild attack) one plot of about 0·7 acre of the same variety was rogued and this had ultimately about four per cent less disease than the control plots.

The removal and destruction of diseased plants on sight is probably one of the most practicable ways of restricting the spread of leaf-curl.

3. The possibility of controlling the vector or vectors by means of spraying, etc. has yet to be explored. While it is out of the question to think of protecting tobacco fields from the insects, it may possibly be feasible to prevent them from having access to nursery-beds. The development of this phase of the control problem must await the results of entomological investigations.

Attention may however be directed to the results set forth in Section IV where seedlings from an insect-proof cage contracted disease, when planted out, just as rapidly as seedlings grown in open nurseries. If anything, the rate of spread, in the early stages, was greater in the case of the former, possibly because the plants (having been grown under protection) were more tender and therefore more attractive to insects.

The possibility of restricting the amount of disease by regulating the time of sowing in relation to the times of the year when the vector is less numerous may be capable of economic application. It will be recalled that tobacco sown early in the monsoon (Section IV) suffered much more severely than that sown later.

4. The breeding of resistant varieties is in general the most efficient method of controlling disease. Strains resistant to various viruses have been secured in a number of crops. In the case of leaf-curl of cotton, a somewhat similar virus disease, Kirkpatrick [1931] reports that " an encouraging start has already been made " in the selection of resistant strains. Storey [1935] however met with no success in his endeavour to discover varieties resistant to tobacco leaf-curl and concluded that there was little prospect of control by this method. Our experience thus far agrees with that of Storey as none of the species or varieties of tobacco which have been grown on a large scale at Pusa shows any considerable resistance. The mosaic-resistant tobacco, Ambalema, was also tried on a small scale but about one-third of the plants contracted leaf-curl. Fig. 2, however, indicates that Pusa H 142 and Adcock are less susceptible than Harrison's Special, Cash or Pusa H 177.

N. plumbaginifolia which, as already mentioned, grows wild does not show any apparent symptoms of leaf-curl and may be really resistant but it is difficult to verify this as, owing to its rosette habit, it cannot easily be grafted. Moreover, the cross with *N. Tabacum* is extremely difficult to accomplish and produces an

F_1 that is completely sterile ; thus even if *N. plumbaginifolia* does possess high resistance to leaf-curl the prospect of transferring this resistance to commercial tobacco by hybridization is remote.

For the present we may conclude that immediate removal of infected tobacco plants and weeding out of alternate hosts are likely to mitigate losses from leaf-curl. The adjustment of sowing time in relation to the periodic distribution of the vector may also alleviate losses. Other measures must await further knowledge.

VIII. SUMMARY

1. Leaf-curl diseases are probably the most serious menace to tobacco cultivation in Northern India. This group of diseases is probably identical with the 'kroepoek' and 'krul' diseases of Java and the 'leaf-curl' of East and South Africa. The literature on the subject is briefly reviewed.

2. Most of the work described was carried out with the cigarette tobacco variety, Pusa H. 142. The technique of grafting and inoculation is described and some of the terms used in describing diseased plants are also explained.

3. The incidence and epidemiology of leaf-curl were studied. While the average amount of leaf-curl plants in a normal year is five to ten per cent, in an epidemic year, such as 1934-35, nearly the whole of the crop may be rendered worthless. Weekly counts of diseased plants showed that fresh infection takes place in varying amounts throughout the growing season. Generally speaking, the rate of spread was greatest at the end of October and in early November when the seedlings were planted out, and there were further large increases in the second half of November and early December. Thereafter there was a decline until the second week of January when no fresh infection at all was recorded. In March however there was again a distinct rise.

In 1935-36 two sowings were done—one in June and the other at the normal time in August. The amount of disease in the former was strikingly high, being about sixty-three per cent more than in the normal-sown crop. This suggests that conditions in the monsoon months are more favourable to the activity of the insect vector.

Leaf-curl symptoms were only extremely rarely noted in seedlings in the nursery-beds, and most of the infection appears to occur after the transplanting operation.

4. Five different types of leaf-curl could be differentiated : these are termed Leaf-curl *A*, Leaf-curl *B*, Leaf-curl *C*, Leaf-curl *D*, and Leaf-curl *X*. The first four are considered to be due to distinct viruses and are named Tobacco Leaf-curl viruses 1-4 while the fifth, Leaf-curl *X*, is thought to be due to mixture of two or more of these viruses, in different combinations.

The characters common to the five types of leaf-curl are : (1) a dwarfing of the plant and reduction of leaf size. (2) curling of the whole or portions of the lamina. (3) the presence of either vein-banding or thickening and greening of the veins, in some or all of the leaves. (4) a condensation of the inflorescence, the flowers of which do not open normally ; the veins of the calyx are usually thickened and 'greened'. (5) the disease is transmissible by grafting but not by juice or by seed.

A and *B* are characterised by the presence of abundant greening and usually also of enations and the reduction in stature is striking. *A*, which has small, severely curled, rugose, much-thickened, brittle and dark-green leaves is readily separated from *B* where the leaves are larger, slightly curled, wrinkled, and light-yellowish green in colour ; they are not thickened or brittle. In *C* and *D* plant-height is not so severely reduced, and enations are completely absent. Both have persistent vein-clearing but this is more intense in *C*. *C* can be easily discriminated from *D* as it usually shows a few green 'stitches' in the older leaves, these being never present in *D*.

Leaf-curl *X* is extremely variable and generally presents an appearance suggesting a mixture of the other types of leaf-curl described. This type was produced synthetically in experiments in which *A*, *B*, *C* and *D* were grafted in pairs in several combinations.

The symptoms produced by the five types of leaf-curl when grafted with *Nicotiana Tabacum* var. Turkish, *N. rustica*, *N. glutinosa*, *Solanum nigrum*, *Lycopersicum esculentum* and *Petunia* sp. are described.

5. The question of control measures is considered.

(*a*) It is pointed out that in East and South Africa and in Java, the removal of ratooning and self-sown tobacco plants and of alternate hosts has given good results, but for the practice to be fully effective in India, more knowledge regarding the alternate hosts is required. At Pusa symptoms (greening, etc.) strongly resembling those of tobacco leaf-curl have been observed on plants of *Zinnia elegans*, *Petunia* sp., *Althaea rosea*, *Hibiscus rosa-sinensis*, *Crotalaria juncea*, and *Scoparia dulcis*.

(*b*) The removal of diseased plants as soon as observed and their replacement with healthy ones is recommended. In the epidemic year 1934-35, plots so treated showed about nineteen per cent less disease than the control plots.

(*c*) The possibility of controlling leaf-curl by protecting nursery-beds (by spraying, etc.) from the vector, is one which is probably worth exploring.

(*d*) The breeding of resistant varieties offers the most satisfactory method of control. At present however the situation in this respect is not very hopeful as none of the species and varieties tested on a large scale at Pusa has shown any considerable resistance. *N. plumbaginifolia* which occurs as a common weed does not show any symptoms and is either resistant or a 'carrier' but it has not yet been possible to verify this by experimental test.

ACKNOWLEDGMENTS

In addition to those whose assistance has been acknowledged in the body of the paper, we are particularly indebted to Dr. Kenneth M. Smith of Cambridge for reading through the manuscript and giving us his valuable advice. We are also grateful to Dr. B. B. Mundkur for reading through the paper and making useful suggestions, to Dr. T. Ahmad for information regarding the influence of meteorological factors on insect incidence, to Mr. Pushkar Nath and Mr. A. Aziz for assistance in making the numerous grafts and inoculations, to the Curator of the Royal Botanic Garden, Sibpur, for identifying certain weeds, and to the Imperial Agricultural Chemist, for kindly arranging to steam-sterilize tobacco seed boxes for us.

Dr. P. S. Hudson of the Imperial Bureau of Plant Genetics, Cambridge, arranged to have portions of Thung's paper translated for us, and we gladly acknowledge this assistance.

REFERENCES

Howard, A. and Howard, G. L. C. (1910). *Mem. Dept. Agric. Ind.* (*Bot. Ser.*) **3**, 1-58.

Hopkins, J. C. F. (1932). *Rhod. Agric. Jour.* **29**, 680-86.

Hornby, A. J. W. (1932). *Nyasaland Prot. Ann. Rept. Dept. Agric.*, p. 41.

Johnson, J. (1927). *Univ. Wisconsin Agric. Expt. Stn. Res. Bull.* **76**, 16 pp.

Kashi Ram (1931). *Ind. Jour. Agric. Sci.* **1**, 455-72.

Kerling, L. C. P. (1933). *Phytopath.* **23**, 175-90.

Khudyna, I. P. (1936). *Bull. No.* **130** *of U. S. S. R., State Institute for Tobacco Investigations, Krasnodar* (Russ. with English summary).

Kirkpatrick, T. W. (1931). *Bull. Ent. Res.* **22**, 323-63.

Kunkel, L. O. (1936). *Phytopath.* **26**, 201-19.

Ludwig, K. (1913). *Bericht. Deutsch. Bot. Gesell.* **31**, 536-43.

Mossop, M. C. (1932). *Rhod. Agric. Jour.* **29**, 869-72.

Roberts, J. I. (1930). *Bull. Ent. Res.* **21**, 169-83.

Salaman, R. N. (1933). *Nature* **131**, 468.

Smee, C. (1932). *Nyasaland Prot. Ann. Rept. Dept. Agric.*, p. 50.

Storey, H. H. (1931). *Nature* **128**, 187-88.

————————(1932). *Rhod. Agric. Jour.* **29**, 186-92.

————————(1935). *East African Agric. Jour.* **1**, 148-53.

Thung, T. H. (1932). *Meded. Proefst. Vorstenlandsche Tabak*, No. 72, 54 pp.

————————(1934). *Idem*, No. 78, 18 pp.

Valleau, W. D. and Johnson, E. M. (1927). *Kentucky Agric. Expt. Stn. Res. Bull.* **281**, 179-253.

THE PULSE BEETLES (*BRUCHIDAE*) OF BURMA

BY

C. C. GHOSH, B.A., F.R.E.S.

Deputy Director of Sericulture, Berhampore, Bengal

(*Formerly Entomologist to the Government of Burma*)

(Received for publication on 20th January 1937)

(With Plates XXXII and XXXIII)

INTRODUCTORY

BEANS fill an important place in Burmese agriculture, especially in the Dry Zone. In 1934-35 the *Season and Crop Report of Burma* showed 1,232,602 acres under peas and beans of various kinds. Of this area 1,081,707 acres occurred in the Sagaing, Mandalay and Magwe Divisions. Rangoon Red beans, Rangoon White beans and gram are exported, the value of these exports in 1928-29 amounting to Rs. 47,67,025. Beans of all kinds form an important part of the diet of the Burmese. Thompstone and Sawyer [1914] have given detailed descriptions of all the principal peas and beans of the Province.

In 1933 and 1934 the writer made an extensive collection of pulses from all over the province. The samples were taken principally from cultivators' threshing floors. They were then grown at Mandalay for two seasons and studies were made of the Bruchid and other insects attacking them in the field and in store. Only observations on the Bruchids are recorded here.

The damage done by Bruchids is sometimes very great. The various types of *Dolichos lablab*, for example, are so severely damaged in store that they cannot be exported. The damage to *Cajanus* is so extensive as to require special selection work to try to evolve resistant types.

The writer is indebted to the Director of the Imperial Institute of Entomology for identification of the beetles. The field and laboratory work has been in charge of Mr. S. D. Williams, Research Assistant.

THE BEETLES

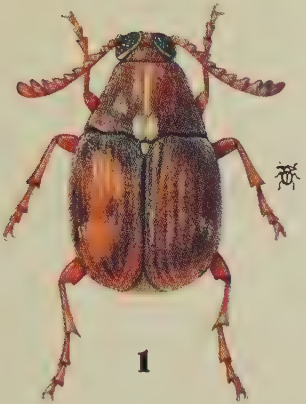
The following beetles have been observed to occur as pests on the seeds of peas and beans of economic importance in the Province. All of them are capable of breeding without taking any food in the adult state. In nature some visit flowers and apparently eat pollen.

Bruchidae (Plate XXXII)	—1. *Bruchus chinensis* L.
	2. *B. analis* F.
	3. *B. phaseoli* Gyll.
	4. *B. albocallosus* Pic.
Anthribiidae	—5. *Araecerus fasciculatus* De G.

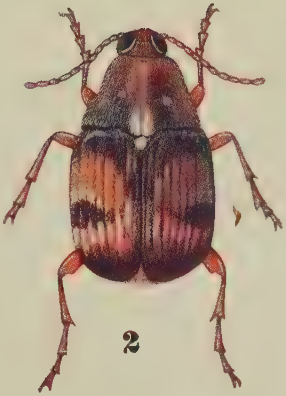
The other Bruchids reared from seeds in Burma are *Bruchus vectabilis* Gyll. on *Sesbania aegyptiaca*, *B. maculipyga* Champ. on *Acacia pennata*, *B. rouyeri* Pic. on *Tephrosia purpurea* and *Caryoborus gonagra* Fab. on tamarind, *Acacia farnesiana* and *A. pennata*. No account of them is however included in this publication.

SOME PREVIOUS RECORDS OF THE BEETLES

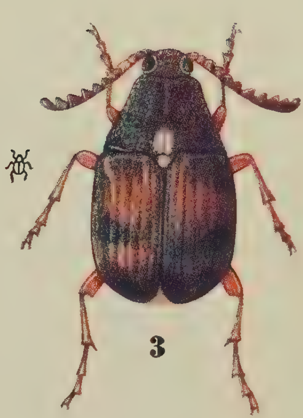
Bruchus chinensis is a cosmopolitan pest. The records of its injurious activities already observed in different countries show that it is considered as a specific pest of cowpeas in the United States of America [Chittenden, 1898] but also occurs there on the common pea, pigeon-pea, lentil, chick-pea, *mung* bean and common white bean [Black, 1922]. In India it is taken to be the common pulse beetle and is definitely recorded [Lefroy, 1909] on peas (*Pisum sativum*), val (*Dolichos lablab*), *D. biflorus*, gram (*Cicer arietinum*), pigeon-pea (*Cajanus indicus*), lentil (*Ervum lens*) and cowpea (*Vigna catjang*). According to Fletcher [1914] this particular species lives only on red gram in the field in Southern India. But Rao [1917] reared it from the pods collected in the field of horse gram (*D. biflorus*), green gram (*Phaseolus mungo*), cowpea, pigeon-pea, *Phaseolus aconitifolius* and cluster beans (*Cyamopsis psoralioides*). At Pusa [Fletcher and Ghosh, 1919] it is recorded on pea, lentil, cowpea, pigeon-pea, gram, horse gram, soy bean, *Lathyrus sativus*, *Vicia faba*, *Phaseolus mungo* and *P. aconitifolius*, two varieties of *Phaseolus radiatus*, locally known as *aghania* and *mungia* and the small field pea, *Pisum arvense* being immune. Black gram (*Phaseolus radiatus*) is recorded as immune also in Tanganyika. In Formosa it is recorded principally on *Phaseolus* spp., *Cajanus indicus*, *Vigna catjang* var. *sinensis*, *Pisum sativum* and *Vicia faba* var. *equina*. In South Africa it is widely spread, infesting cowpeas, and is also recorded on cotton bolls and seeds and on sorghum millets in Africa. In



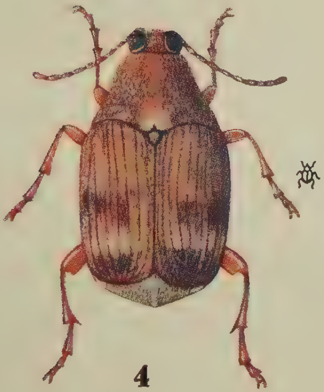
1



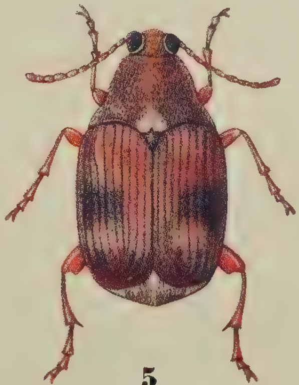
2



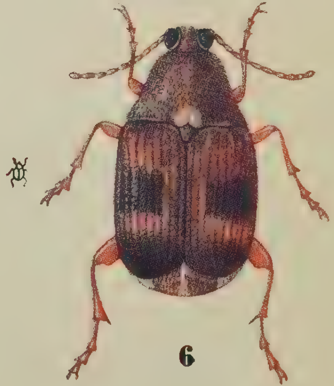
3



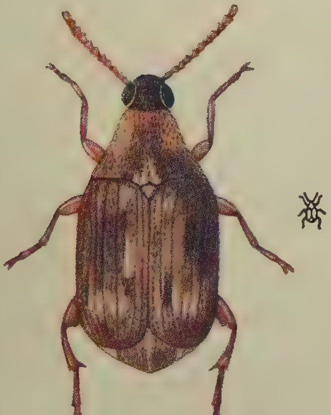
4



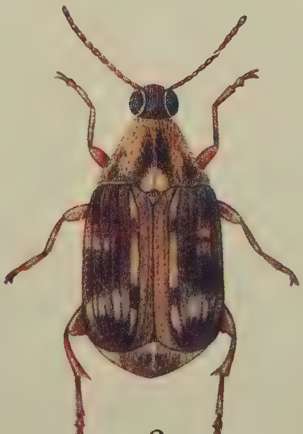
5



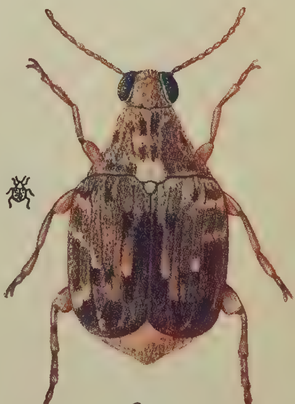
6



7



8



9

FIG. 1, 2, 3. *Bruchus chinensis.*

FIGS. 4, 5, 6. *Bruchus analis.*

FIGS. 7, 8. *Bruchus phaseoli.*

FIG. 9. *Bruchus albocallosus.*

Hawaii it frequently attacks pigeon-peas and adults have also been bred from *Phaseolus articulatus*, *P. aureus*, *Vigna sinensis*, *Cajanus indicus*, *Glycine hispida*, *Cicer arietinum*, *Vicia faba* and *Pisum sativum*. In Java *Arachis hypogaea* and *Cajanus indicus* are said to suffer severely.

Bruchus analis is recorded on cowpea and dried pulses from India and Mysore.

Bruchus phaseoli is apparently the same species as recorded as *Bruchus* sp. on *lablab* in Madras [Rao, 1917] and on beans in store at Pusa [Fletcher and Ghosh, 1919] and as *Bruchus theobrome* in South India [Fletcher, 1914]. It is also recorded from Jamaica, Hawaii, Brazil, France and Italy and is said to have been introduced into Russia in broad beans. It attacks field peas in Jamaica. In Hawaii the usual host is *Dolichos lablab* but eggs have also been found on lima beans (*Phaseolus lunatus*) and in captivity it has been bred from pigeon-pea, cow-pea, soy bean, chick-pea (*Cicer arietinum*); *Phaseolus angularis*, broad bean, *mung* bean (*Phaseolus aureus*) and common pea.

Bruchus albocallosus has not been recorded in publications available to the writer.

Araecerus fasciculatus is a cosmopolitan insect. It breeds in areca nuts in India and it or allied species have been bred from seeds of old unpicked dry cotton bolls and dry chilli pods [Lefroy. 1909] and from fruits of Persian Nim trees (*Melia*) at Coimbatore [Ayyar, 1921]. Beeson records it on *Areca catechu* and on some *Papilionaceae*. It is described as Coffee Beetle or Coffee Bean Beetle and considered a pest on coffee in the Dutch East Indies and also has been recorded on coffee in Brazil and Trinidad, on *Curcuma* pods in Hawaii, on cacao, sweet potato, sugarcane and French beans in Brazil, on pods of *Tephrosia candida* in Ceylon and the Dutch East Indies, on *Crotalaria*, *Indigofera*, *Bauhinia* and *Cajanus indicus* in Ceylon and on cotton in Hawaii, Africa and Brazil. In the store it is recorded on cacao in Nigeria, Brazil, Germany, Tanganyika and West Africa, on coffee in Brazil, Belgium, Cuba and Madagascar, on maize in Bermuda, Sierra Leone and Brazil, on rice in Brazil, on ginger in Sierra Leone, on sweet potato in Alabama, on cotton in U. S. A., on nutmeg and mace in Grenada, on Brazil nuts (*Bertholletia excelsa*) in Malaya, on coconuts and palm nuts in the Belgian Congo, and on dried fruits in Brazil. It is also recorded as intercepted on avocado seeds, nutmeg, various spices, dried vegetables and dried yams in the U. S. A., on beans in the Philippines, on cassava roots in Germany, on cacao beans in Britain and on seeds of *Guarea thomsoni* in Uganda. In the Philippines it is said to carry *Diplodia*, causing rot in stored roots and fruits. In Bermuda it is associated with the Black Tip disease of bananas. In New South Wales it is declared as a pest under the Pest Act.

OCCURRENCE OF THE BEETLES AS OBSERVED IN BURMA

In the following statement the natural occurrence of the four species of *Bruchidae* as observed in the field or in the store is noted and the intensity of damage observed is indicated as major (M) or minor (m).

Pulses			Attacked	
Common English name	Scientific name	Burmese name	In the field by	In the store by
Pigeon-pea	*Cajanus indicus*	*Pe-sin-ngon*	*B. chinensis* (M) *B. albocallosus* (m) *B. analis* (m).	*B. chinensis* (M). *B. analis* (m).
Large white bean	*Dolichos lablab* var. *lignosus.*	*Pegyi*	*B. phaseoli* (M) *B. chinensis* (m) *B. analis* (m).	*B. phaseoli* (M). *B. analis* (m).
	D. lablab var. *typicus*	*Maung-ma-khaw-pe*	*B. phaseoli* (M)	*B. phaseoli* (M). *B. analis* (m).
	D. lablab	*Pe-seikgyo*	*B. phaseoli* (m) *B. chinensis* (m).	*B. phaseoli* (m).
	D. lablab	*Shwe-yinma*	*B. phaseoli* (m)	*B. phaseoli* (M).
Horse gram	*Dolichos biflorus*	*Pebizat*	*B. Chinensis* (m)	*B. chinensis* (m).
	Dolichos sp.	*Dungghaw*	...	*Araecerus fasciculatus* (m). *B. chinensis* (m).
Moki Lima bean	*Phaseolus lunatus*	*Htawbape*	...	*B. chinensis* (m). *B. phaseoli* (m).
Rangoon small white bean.	Do.	*Pebyugale*	...	*B. phaseoli* (m).
Sivapole lima	Do.	*Htawbape*	...	*B. phaseoli* (m).
E. B.'s No. 2730	Do.	Do.	...	*B. phaseoli* (m).
Henderson's bush lima.	Do.	Do.	...	*B. phaseoli* (m).
Gram	*Cicer arietinum*	*Kalape*	*B. chinensis* (m) *B. analis* (m)	*B. chinensis* (m). *B. analis* (m).
Black gram	*Phaseolus mungo*	*Matpe*	*B. analis* (m)	*B. analis* (m).
Green gram	*P. radiatus*	*Pedi, Pedisein Penauk.*	*B. analis* (m) *B. chinensis* (m)	*B. analis* (m). *B. chinensis* (M).
Pea	*Pisum sativum*	*Sadawpe*	*B. chinensis* (m)	*B. chinensis* (M).
Cowpeas	*Vigna catjang*	*Pelun, Peletkauk, Pebokeshe, Pedauk, Thonlape,* etc.	*B. analis* (m) *B. chinensis* (m)	*B. analis* (M). *B. chinensis* (M).
Soy beans	*Glycine hispida*	*Pengapi.*	*B. chinensis* (m)	*B. chinensis* (m). *B. analis* (m).
Lobia or Indian Rice bean.	*Phaseolus calcaratus*	*Peyin* and *Khawtumpe*	*B. chinensis* (m)	*B. chinensis* (m). *B. analis* (m). *B. phaseoli* (m).
Chickling Vetch	*Lathyrus sativus.*	*Pechinboung*	...	*B. chinensis* (M).
Sweet peas	*Lathyrus odoratus*	*Peleikpya*	*B. chinensis* (m)	*B. chinensis* (m).
Sword bean	*Canavalia ensiformis*	*Pedalat*	*B. albocallosus* (m)	*B. analis* (m).
Cluster beans *	*Cyamopsis psoralioides*	*Pewalee*	...	*B. chinensis* (m).

---

* This bean being wholly used as a vegetable its seeds were not sent in. It is however known to be attacked in the store.

TESTING OF IMMUNITY OR RESISTANCE OF SEEDS BY ARTIFICIAL INFECTION

Out of the four Bruchids, three, *viz.*, *B. chinensis*, *B. analis* and *B. phaseoli* have been observed to breed in dry seeds in the store. The statement given in the appendix shows the results of trials to test the immunity or otherwise against these three store pests of the various kinds of peas and beans.

NOTE ON LIFE-HISTORY AS OBSERVED AT MANDALAY

Bruchus chinensis

This beetle lays a single egg at a place but usually there are several eggs on the same pod or seed. The eggs are glued on the surface and are elongate oval in shape, the ends being rather tapering and not exactly rounded. The surface of the egg is smooth and shiny and its colour is pale yellow, a dark spot appearing at one end before hatching. The grub on hatching works its way into the pod or seed. On the pod a hole is visible under a lens below the empty egg-shell which is surrounded by dust thrown out by the grub while it works its way in. On the seeds the empty egg-shell usually gets filled with dust and loses its shininess. The grub feeds and develops inside the seeds even when pods are infested and if the infested seeds remain inside unopened pods the resultant beetle on maturity emerges through a hole in the pod in the same way as from seeds in the store.

The statement below shows the number of eggs laid by a female and the duration of their life-cycle. The pairs emerged on 12th October morning.

Date	Pair No. 1				Pair No. 2				Pair No. 3				Remarks
12-10-1934 . . .	1	1	..	1	1	2	1	2	5	7	5	3	The four figures under each pair on the same date show eggs laid on the four different *Cajanus* seeds supplied.
13-10-1934 . . .	4	7	5	5	5	4	6	5	7	6	4	9	
14-10-1934 . . .	2	2	1	3	2	4	3	3	3	1	3	...	
15-10-1934 . . .	1	1	1	1	1	2	1	1	...	...	...	1	
16-10-1934 . . .	1	...	...	...	2	...	1	...	...	...	...	...	
					1 dead								
17-10-1934 . . .	1 dead				...				1 dead				
18-10-1934 . . .	1 dead				1 dead				1 dead				
Total No. of eggs . .	38				47				55				
Total No. of beetles which emerged between 5-11-1934 and 9-11-1934, the period of life cycle being 23 to 27 days.	14				17				10				
No. of seeds on which eggs laid.	16				18				12				

This works out to less than one beetle per seed. Several, up to about half a dozen beetles, have however been observed to breed out of a single seed.

Apart from temperature the nature of the seed on which the beetle bred was observed to have an effect on the duration of the life-cycle. On *Cajanus indicus* seeds, which suffer most from this beetle, the life-cycle occupied a maximum of forty-three days in January-February and a minimum of twenty days in March-April. In the latter months the period on Behrun soy bean (*Glycine hispida*) was forty-one days, on lentil (*Lens esculenta*) thirty-four to forty-one days and on different strains of peas twenty-four to thirty-four days. On seeds which were not congenial to the beetle but on which it would live and breed with difficulty as was evident from the small size of the offspring, the life-cycle was very much lengthened. Thus on two lima beans (*P. lunatus*) it took fifty to fifty-three days between January and March and on a Burmese lima bean (*P. lunatus*) it took 189 days between January and July.

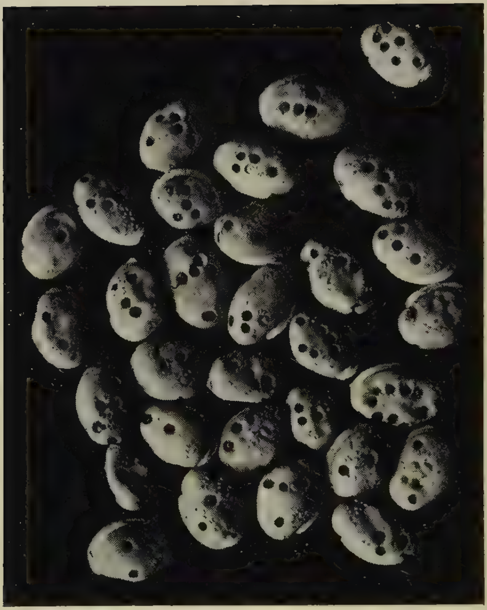
Bruchus analis

Beetles mate freely and oviposit soon after emergence. Males eagerly follow females and attempt mating and usually have several failures before success. When successful the male becomes passive and lets go its hold of the female and holds the legs, especially the hind pair raised behind the back and appears to sit on its own hind quarters behind the female. During mating the female is usually quiet and at times throws up her hind legs in air over the back. Towards the end of mating she kicks the male with her hind legs and frees herself from him with some effort. The mating periods observed of six pairs about 9 A.M. were 7, 9, 5, 6, 6 and 6 minutes. When the six coupling pairs were isolated the males attempted mating again after separation but without success.

Below are shown the oviposition records of six pairs which emerged from cowpeas harvested from the field. Each pair was provided with four seeds every day and the figures show the number of eggs laid on each seed. Only one beetle however bred out of each seed.

Bruchus analis *on pelon seeds*

Date	Pair No. 1				Pair No. 2				Pair No. 3				Pair No. 4				Pair No. 5				Pair No. 6			
12-1-1934	6	4	5	7	3	3	2	3	3	3	5	5	6	4	4	4	1	2	—	1	4	3	5	6
13-1-1934	2	3	2	2	2	3	3	2	2	2	3	3	4	4	2	3					3	4	5	5
14-1-1934	2	2	2	3	4	3	2	2			1		3	4	2	3					2	1	1	1
15-1-1934	1	1			4	3	4	2	4		1		5	4	5	5	5	4	5	3	2	3	4	1
16-1-1934					1	1	1	1					1	1	1	2	2	1	2	3			1	
17-1-1934					1	2	1						1	1	2	1								
18-1-1934	1 dead				2	1							2	4	4	2	1							
19-1-1934					2	1							2	2		1								
20-1-1934	1 dead												1 dead								1 dead			
25-1-1934					2 dead				2 dead				1 dead				2 dead				1 dead			
Total No. of eggs	42				58				32				89				30				51			
Total No. of beetles emerged between 21-2-1934 and 17-3-1934, the period of life cycle being 40 to 65 days.	7				10				7				19				5				10			
Total No. of seeds on which eggs laid.	14				27				11				31				12				17			



The eggs of *Bruchus phaseoli* on *Dolichos* seeds damaged by the breeding of the same beetle in store.

One coupling pair which emerged from cowpea seeds on 10th January 1934 was isolated and provided with ten cowpea seeds at about 10 A.M. Eggs were observed to have been laid by 2 P.M. The female died on 16th January and the male on 22nd January. The number of eggs laid on the ten seeds were 2, 3, 3, 8, 4, 2, 4, 4, 4 and 5 or 39 in all. But only one beetle emerged from each seed. The seeds were soaked in water and dissected. Only one grub had fed in each except in the one with eight eggs in which there was a pupa in a second chamber. This apparently was an exception. Usually only one beetle develops in a single seed.

Eggs are deposited singly at a place on the surface of seeds and pods.

As in the case of *Bruchus chinensis* the nature of the seed on which the beetle bred affected the duration of its life-cycle. On cowpeas in which the beetle seemed to be most at home the minimum period of the life-cycle was nineteen days in March-April and the maximum period in December-January was forty days. On different strains of *Cajanus indicus* the life-cycle took twenty-six to as much as ninety-nine days between December and March and on different strains of *Dolichos* 18 to 107 days between January and June. On peas it did not vary very much occupying about thirty-one to thirty-three days in March-April-May. While on *Phaseolus mungo* it ranged between twenty-eight and fifty-two days in February-March.

Bruchus phaseoli

Eggs are laid on green pods on the plant usually in clusters and several clusters may be laid on the same pod. Of three pods of *maung-ma-khaw-pe* (*Dolichos lablab* var. *typicus*) picked at random on 2nd December 1933 one had five clusters, the 2nd and 3rd had three and one respectively. There were three to eight eggs in a cluster. The eggs are almost cylindrical and elongate oval in shape with a shiny surface and in colour at first pearly white changing to light yellow. They are heaped together at a place and the heap held together and held on the surface of the pod by a gummy substance of the same colour as the eggs. Eight eggs measured 0·7×0·35, 0·6×0·35, 0·72×0·34, 0·735×0·39, 0·615×0·315, 0·715×0·4, 0·72×0·39 and 0·705×0·315 mm. The mode of oviposition is different in the store. The beetles which developed from the above pods had access to the same dry pods as well as to dry seeds. On the seeds as well as on the bottom and walls of the glass jar eggs were laid singly but on the pods both singly and in clusters. On seeds in the store eggs are laid singly although there may be many eggs on the same seed (Plate XXXIII). Also in the store dry seeds have been observed to be preferred for oviposition to green pods supplied.

The beetles mate soon after emergence and commence oviposition on the second day. Six pairs which emerged from *pegyi* (*Dolichos lablab*) seeds and mated on 9th October 1934 were isolated and provided with two *pegyi* seeds every day. No food was provided. The statement below shows the period of oviposition, number of eggs laid by each female, the number laid on a single seed in a day, the period of adult life and the number of eggs which developed into adult stage.

Date	Pair No. 1		Pair No. 2		Pair No. 3		Pair No. 4		Pair No. 5		Pair No. 6	
11-10-1934	11	13	6	7	12	14	12	20	18	15	16	18
12-10-1934	6	5	8	9	11	7	9	13	32	18	3	10
13-10-1934	1	2	12	14	9	4	12	2	14	15	10	7
14-10-1934			5	3			4	3	11	6	1	3
15-10-1934	1 dead		6	5	1 dead		1	2	2	4	2	2
16-10-1934	1 dead		4	3	1 dead		4		1 dead		1 dead	
17-10-1934			1 dead				1 dead		1 dead		1 dead	
20-10-1934			1 dead				1 dead					
Total No. of eggs . . .	38		82		57		82		137		72	
No. of adults which developed from the eggs and emerged between 3-11-1934 and 15-11-1934, the period of life cycle being 23 to 35 days.	24		50		36		51		58		17	
No. of seeds from which these beetles bred.	6		12		6		11		11		10	
Average No. of beetles from a seed.	4		4		6		4·6		5·2		1·7	

Bridwell has recorded fourteen beetles from a single seed of *Dolichos lablab* in Hawaii. As will appear from the holes in the seeds in Plate XXXIII many beetles usually breed in a seed. From two seeds in the pod with five egg-clusters mentioned above as collected from the field thirteen beetles bred out. On this account both in field and store infestation is attended with severe damage.

As in the case of *B. chinensis* and *B. analis* the nature of seed on which the beetle bred had great effect on the duration of the life-cycle. On *Dolichos* spp., the favourite food of this beetle, the life-cycle took eighteen to thirty-eight days, on cowpeas it ranged from twenty-four to forty-one days, on different strains of *Phascolus lunatus* from fifty-three to 122 days, on *Cajanus indicus* twenty-five to thirty-eight days and on peas thirty to forty-one days. The offspring bred out of *P. lunatus* seeds were much smaller in size and paler in colour than the parents and were considered in the British Museum to be a variety of this species.

Bruchus albocallosus

It has been met with only in pods collected from the field. Eggs are apparently laid on the pods. Grubs on hatching bore into the seeds in which they feed and develop. The beetles on emergence issue out of the seeds but cannot or do not cut out of the pods and wait until the pods are broken open. On 30th March adult beetles were found in dry *Cajanus* pods left on the plants ready to fly away when the pods were broken open. Some of these pods were left unopened till 10th May when a few beetles were found still alive and waiting inside them.

GENERAL OBSERVATION AS REGARDS DAMAGE.

Out of the four Bruchids the two most injurious are *Bruchus chinensis* on pigeon-peas (*pesinngon*) specially and also on cowpeas (*Vigna catjang*) and *B. phaseoli* on the beans proper, *viz.*, *Dolichos lablab* and its varieties (*pegyi*, *maung makhawpe*, *peseikgyo*, *shweyinma* and others).

The third one *B. analis* is a pest on *Phaseolus mungo* and *P. radiatus* (*pedi*, *pedisein* and *matpe*) and the cowpeas (*Vigna catjang*). Unlike the foregoing two, many of which breed in a single seed this species breeds at the rate of only one in a seed and is therefore less injurious.

The fourth, *B. albocallosus*, occurs in small numbers only in the fields on pigeon-peas (*Cajanus indicus*) and can hardly be considered as a pest.

The Anthribiid beetle, *Araecerus fasciculatus* cannot be considered as a pest.

By comparing the statements under natural occurrence and immunity tests it will be observed that although *B. chinensis* failed to breed in *pegyi*, *Dolichos lablab* and its varieties, in the immunity tests it occasionally occurs on this bean in the store as well as in the field. The real and very injurious pest on this bean is *B. phaseoli*. The seeds are badly infested in the field and holes due to emergence of the beetles appear in the seeds soon after harvest. The beetles are also very prolific breeders in the store. Therefore unless properly looked after the seeds are liable to serious damage in a very short time. This is a serious obstacle in the export of this bean. This bean is again the most liked in the country as food and therefore extensively grown and stored. How damage to it can be prevented or at least minimised is discussed under control measures below.

The *P. lunatus* beans are practically immune to all the three injurious beetles and out of them again the type *peni* is totally immune. *B. analis* has not bred in any of sixty-six different *lunatus* samples exposed to it, *chinensis* has bred only in three out of thirty-three samples exposed to it and *phaseoli* in eleven out of thirty-three samples exposed to it. *B. phaseoli* is more at home with these seeds than *chinensis*. That these seeds, however, were not quite congenial to both these species which apparently bred in them with difficulty, will be evident from the fact that the period of their life-cycles on these seeds were lengthened, in some cases enormously, and that the offspring were smaller than the parents. On one variety of *P. lunatus* locally known as *pe-ga-dipa* the life-cycle of *chinensis* took 189 days, and that of *phaseoli* 122 days. Kidney beans (*P. vulgaris*) are immune to all the three and so are Chinese potato and Goa beans.

In the immunity tests soy beans show good results but are liable to infection though slight both in the field and the store.

The cowpeas are liable to damage by all the three beetles, though only *B. chinensis* and *B. analis* have been observed to occur naturally. In the field again more *analis* has occurred on them than *chinensis*.

The pigeon-peas are about the same as cowpeas in their relationship with the three beetles. But *B. chinensis* is the most common pest on them both in field and store. They are moreover liable to be attacked by *B. albocallosus* in the field but this beetle has not so far been observed to be of much importance.

Neither gram nor peas have so far been observed to be damaged severely, though they are not immune.

Control measures

As will appear from the foregoing observations the problem of prevention or control of damage by the pulse beetles does not affect all kinds of peas and beans. But the kinds affected are important ones and in the case of all of them infestation commences before the seeds are harvested. Not all but some of the seeds are attacked in the field. All the three injurious species of beetles (*B. chinensis*, *B. analis* and *B. phaseoli*) thus find their way into the store with the infested seeds and as all are capable of breeding in the store the sound seeds are attacked later on in the store where really the greatest damage takes place.

Damage in the store can be prevented (1) if the seeds already infested can be treated so as to kill the insects present in them before storage by the application of an insecticide or heat or (2) if the infested seeds can be separated out and only sound ones stored as in the case of large bean seeds by steeping in water (see below). Then storage must be under conditions which will prevent access of any beetles from outside.

Whatever measures are adopted they must be adopted soon after harvest as the beetles emerge from the infested seeds very soon and continue breeding in the dry seeds.

Effect of heat

When the majority of the peas and beans are harvested hot sunshine is available. If the seeds are sunned thoroughly for several days by being spread out in thin layers on a smooth surface plastered with mud or cow-dung which is heated by the sun, a good deal of damage is saved. Solar heat can be made use of by all cultivators. This is an essential treatment at harvesting time.

Artificial heat affects different kinds of seeds, especially their germination, in different ways. A preliminary trial in the laboratory showed that peas (*Pisum sativum*) lost germinative power when kept at 70°C. for fifty minutes, *pebyugale* (small white bean—*Phaseolus lunatus*) lost it when kept at 60°C. for twenty minutes and *pegyi* (*Dolichos lablab* var. *lingnosus*) lost it when kept at 80°C. for ten minutes. The grubs breeding inside the large *pegyi* seeds were not all killed at this temperature. Arrangement for application of artificial heat involves expenditure and is not within the means of ordinary cultivators.

Effect of cold

A temperature of 40°F. lengthened the life and inhibited breeding of *B. analis* adults. Below are shown the results of keeping a number of adult *analis* beetles at this and room temperature (about 81°F.)

No. of beetles	Date of emergence (January (1934)	Kept at temperature	Seeds supplied	Total No. of eggs laid	Beetles died between	Life in days	Eggs hatched or not (Under observation for 185 days in cold store)
7	18	40°F .	*Maung-ma-khaw-pe* .	13	2nd-16th Feb. 1934 .	15—29	No.
7	18	Room	Do. . .	275	26th Jan. to 2nd Feb-1934.	8—15	Yes.
8	10	40°F.	Pigeon-pea . . .	8	1st-26th Feb. 1934 .	22—47	No.
8	10	Room	Do. . . .	41	18th-25th Jan. 1934	8—15	Yes.
8	10	40°F.	Cowpea . . .	25	1st Feb. to 8th Mar. 1934.	22—58	No.
8	10	Room	Do. . . .	99	18th-25th Feb. 1934	8—15	Yes.
8	10	40°F. .	Green gram . . .	13	15th Feb. to 15th Mar. 1934.	36—65	No.
8	10	Room	Do. . . .	86	25th Jan. to 1st Feb. 1934.	15—22	Yes.

Storage of peas and beans in cold temperature is hardly a practicable proposition in this country.

Effect of steeping in water

Seeds in which grubs of *B. phaseoli* feed can, to a great extent, be separated from sound seeds by a period of steeping in water. A lot of *pe-seik-gyo* (*Dolichos lablab* var. *typicus*) seeds harvested and brought in for storage was steeped in water on 24th January 1934 for varying periods and the results are shown below. Badly corroded seeds float when thrown into water. Infested seeds which are not so much corroded do not float but swell in size apparently by soaking water through the hole of entrance of the grubs. The longer the grubs have been feeding inside the quicker is the swelling. Some infested seeds, apparently freshly infested, did not swell even after four hours under water while some swollen seeds did not seem to have been infested.

Seeds	No. of seeds	Date on which beetles, *B. phaseoli*, began to emerge from the seeds	Total number of beetles which emerged
A. which floated on being put in water .	8	31st January 1934 .	54
B. which swelled after 1 hour in water .	76	5th February 1934 .	61
C. which swelled after 2 hours in water .	25	7th February 1934 .	6
D. which swelled after 3 hours in water .	14	12th February 1934	2
E. which swelled after 4 hours in water .	17	7th February 1934 .	1
F. which did not swell after 4 hours in water	344	16th February 1934	11
	484		135

It would seem that about one to one-and-a-half hours of steeping will eliminate the major portion or practically all of the infested seeds in a lot. This method is easily applicable to seeds of *Dolichos lablab* and its varieties.

Storage under sand

The method of storing wheat, gram, etc., under sand as recommended by Fletcher and Ghosh [1919] is applicable to lots ordinarily stored by cultivators of almost all kinds of seeds. The layer of sand inhibits breeding and also is an effective barrier against all kinds of stored grain pests.

The method of spreading a sheet of cloth above the seeds and below the layer of sand has not been found quite effective in practice in the case of large seeds like *pegyi* (*Dolichos lablab*), etc., the large size of which leaves sufficient room in the interspaces to the beetles to move about among the mass of seeds and oviposit on them. In the case of these seeds the methods should be thorough sunning, then steeping in water and the unswollen seeds stored under dry sand taking care to add sand on top when it settles so as to have the seeds always covered.

Storage under sand although not an ideal method, is simple and within the means of all and at the same time effective. No other method can at present be suggested to replace it.

REFERENCES

Ayyar, T. V. R. (1921). *Proc. 4th Ent. Meeting, Pusa*, p. 33.

Black, E. A. (1922). *U. S. A. Dept. Agric. Farmers Bull.* No. **1275**.

Bose, R. D. (1932). *Ind. Jour. Agric. Sci.* **2**, pt. 6.

Chittenden, F. H. (1898). *Year Book of U. S. A. Dept. Agric.*, p. 234.

Fletcher, T. B. (1914). " Some South Indian Insects ", p. 307.

Fletcher, T. B. and Ghosh, C. C. (1919). *Proc. 3rd Ent. Meeting Pusa* (also published as *Pusa Bull.* No. **111**).

Howard, A.; Howard, G. L. C. and Khan, A. R. (1915). *Mem. Dept. Agric. Ind.* (*Bot. Ser.*) **7**, No. 7.

Howard, G. L. C. and Khan, A. R. (1928). *Mem. Dept. Agric. Ind.* (*Bot. Ser.*) **15**, No. 2.

Lefroy, H. M. (1909). " Indian Insect Life " pp. 350 and 380.

Rao, Y. R. (1917). *Proc. 2nd Ent. Meeting, Pusa*, p. 57.

——————— (1919). *Agric. Res. Inst., Pusa, Bull.* No. **89**, p. 23.

Thomstone, E. and Sawyer, A. M. (1914). *Burma Dept. Agric. Bull.* No. **12**.

NOTE.—Where no reference is mentioned it is to be taken as from *Review of Applied Entomology, Ser. A*.

APPENDIX

Immunity tests

The varieties and types of peas and beans locally recognised and usually distinguished by local names on account of difference in colour, shape, size, etc., are noted separately, as also the variations observed after growing them in the experimental area noted as A, B, etc. Where the seed was not attacked it is indicated by N and attack is indicated by a dash. In all cases eggs were laid plentifully by all the three species of beetles.

Pulses			Beetles			Remarks
Common English name	Scientific name	Burmese name or other description	B. *chinensis*	*B. analis*	*B. phaseoli*	
Lablab bean	*Dolichos lablab* var. *typicus*.	*Maung-ma-khawpe*. (Black).	N	—	—	*B. analis* has been observed to occur on it in the store.
		Do. (Black A)	N	—	N	
		Do. (Mottled)	N	—	—	
		Do. (Mottled A)	N	N	—	
		Pe-seikgyo	N	—	—	Pods flat.
		Do.	N	—	—	Pods constricted and somewhat twisted.
	D. lablab var.	*Shweyinma*	N	—	—	Seeds white.
	Dolichos sp.	*Dungghaw*	N	—	—	Seeds dark brown.
Horse gram	*D. biflorus*	*Pebizat*	—	N	N	
Rangoon small white bean.	*Phaseolus lunatus*.	*Pebyugale*	N	N	—	Seeds white. *B. phaseoli* offspring much smaller than parents.
		Pebyugale (A)	N	N	N	Seeds light brown.
Rangoon red bean.	*Phaseolus lunatus*.	*Pegya*	N	N	N	Seeds red.
		Pegya (A)	N	N	N	Seeds light brown.
		Pegya (B)	N	N	N	Seeds dark brown.
		Pegya white	N	N	—	*B. phaseoli* offspring smaller than parents.
		pegya white (A)	N	N	N	Seeds light brown from white-seeded variety sown.
		Pegya brown	N	N	N	
		Do. (A)	N	N	N	Seeds light brown from *pegya* brown.
		Do. (B)	N	N	N	Seeds dark brown.
		Do. (C)	N	N	N	Seeds purple coloured.
Moki lima bean	Do.	*Htawbape*	N	N	—	Seeds white. *B. phaseoli* offspring much smaller than parents.
Do. (A)		Do.	N	N	N	Seeds reddish from original white seeds.
Do. (B)		Do.	N	N	N	Seeds dark red.

APPENDIX—*contd.*

Pulses			Beetles			Remarks
Common English name	Scientific name	Burmese name or other description	*B. chinensis*	*B. analis*	*B. phaseoli*	
Sivapole lima	*Phaseolus lunatus*	*Htawbape*	—	N	—	*B. phaseoli* and *B. chinensis* offspring smaller than parents.
Lima bean E. B's No. 2730.	Do.	Do.	N	N	N	Seeds white.
Lima bean E.B's. No. 2730(A).	Do.	Do.	N	N	N	Seeds red from original white seeds.
Do. (B)			N	N	N	Seeds dark red.
Hendersons' bush lima	Do.	Do.	N	N	—	Seeds white. *B. phaseoli* offspring smaller than parents.
Do. (A)			N	N	N	Seeds reddish from original white.
Do. (B)			N	N	N	Seeds reddish brown.
Do. (C)			N	N	N	Seeds dark brown.
Do. (D)			N	N	—	Seeds purple. *B. phaseoli* offspring very small.
Lima bean E.B's No. 2946.	Do.	Do.	—	N	—	Seeds white. *B. phaseoli* & *chinensis* offspring much smaller than parents.
Burmese lima bean.	Do.	*Peni*	N	N	N	Seeds red. This variety is totally immune.
Do. (A)			N	N	N	Seeds light red from original red seeds.
Burmese lima bean.	Do.	*Pekadiba*	N	N	—	Seeds red. *B. phaseoli* offspring much smaller than parents.
Do.	Do.	*Pekadiba* (A)	N	N	—	Seeds light red from original red deeds. *B. phaseol* offspring much smaller than parents.
		Do. (B)	N	N	—	Seeds brownish red. *B. phaseoli* offspring much smaller than parents.
		Do. (C)	N	N	—	Seeds purple. *B. phaseoli* offspring much smaller than parents.
		Do. (D)	—	N	N	Seeds dark purple. *B. chinensis* offspring smaller than parents.
Do.	Do.	*Pe-leikpya*	N	N	N	Seeds black with white markings.
Do.	Do.	Do. (A)	N	N	N	Seeds with pinkish tinge from original black.
Madagascar bean	Do.	*Pebyugyi*	N	N	N	
Green gram	*Phaseolus radiatus.*	*Pedi or pedisein*	N	—	N	Colour dull green.
		Do. (A)	—	—	N	Colour shining.

APPENDIX—*contd.*

Pulses			Beetles			Remarks
Common English name	Scientific name	Burmese name or other description	B. *chinensis*	*B. analis*	*B. phaseoli*	
Black gram	*Phaseolus mungo*	*Matpe*	N	—	N	Seeds black.
		Do. (A)	N	—	N	Dull greenish brown.
		Do. (B)	N	—	N	Brownish green.
		Matpe small seed	—	—	—	*Matpe* is immune to *chinensis.* But this small seeded type has been observed to be liable to attack by all the three beetles. *B. chinensis & phaseoli* apparently bred with difficulty the offspring being small.
Indian rice or lobia bean.	*Phaseolus calcaratus.*	*Peyin*	—	N	N	Seeds greenish yellow. *B. chinensis* offspring smaller than parents. All the three beetles have been observed to occur on it in store in small numbers.
Gram	*Cicer arietinum*	*Kalape* (Burmese)	N	—	N	
		Do. (Karachi)	—	—	—	
Kidney, French or Haricot bean.	*Phaseolus vulgaris.*	*Bosape* white	N	N	N	
		Do. yellow	N	N	N	
		Do. purple	N	N	N	
		Do. reddish	N	N	N	
		Do. bluish black	N	N	N	
		Do. Canadian Wonder Refugee	N	N	N	
		Do. Stringless green pod.	N	N	N	
		Do. Hudson's long pod.	N	N	N	
		Do. Curries Black Wax.	N	N	N	
		Do. Otenashi bean	N	N	N	
Chinese potato	*Pachyrhizus angulatus.*	*Pe-sein-sa*	N	N	N	
Goa or Kaffir bean.	*Psophocarpus tetragonolobus*	*Pemyit*	N	N	N	
Lentil	*Ervum lens*	*Pe-yaza*	—	N	N	
		Do. (A)	—	N	N	
Chikling vetch	*Lathyrus sativus*	*Pe-chinboung*	—	N	N	
Pea	*Pisum sativum*	*Sadawpe*	—	—	N	Seeds big, light cream coloured. Only 3 *analis* offspring bred out of 70 eggs on 20 seeds.
		Do. Small	—	N	N	Seeds smaller than above with a black mark near hilum.
		Do. Tasmanian Blue pea.	N	—	—	Seeds blue.

APPENDIX—*contd.*

Pulses			Beetles			Remarks
Common English name	Scientific name	Burmese name or other description	*B. chinensis*	*B. analis*	*B. phaseoli*	
		Sad awpe Japanese Marrow fat.	—	N	N	
		Do. Chinese pea	—	N	N	
		Do. Dutch Marrow fat.	N	—	—	
		Do. Small Dutch round.	—	N	N	
Pea	*Pisum arvense*	No. A 14	—	N	—	Imperial Economic Botanist's number.
		No. A 14 (A)	—	N	—	Lighter coloured from above.
Sword bean	*Canavalia ensiformis.*	*Pedalat*	N	N	N	
Soy bean white	*Glycine hispida*	*Pengapi*	N	N	N	Burmese variety. Seeds yellow. *B. chinensis* has been observed to occur on it in field as well as in store and *analis* in store but in small numbers.
Do. black	Do.	*Peme*	N	N	N	Burmese variety. Seeds black.
Do. large green.	Do.	*Pengapi*	N	N	N	Seeds from China through Economic Botanist, Burma.
Do. large pearl.			N	N	N	Do.
Do. little pearl.			N	N	N	Do.
Soy bean Behrum.			—	N	N	Do.
Cowpea	*Vigna catjang*	*Pelon* (brown)	—	—	—	
		Do. (reddish)	—	—	—	
		Do. (whitish red)	N	—	—	
		Do. (light red)	—	—	N	
		Do. (white)	—	—	—	
		Yarpe	—	—	—	*B. chinensis* offspring smaller than parents.
		Pe-gyaung	—	N	—	*B. phaseoli* offspring smaller than parents.
		Shan pegya	—	—	—	*B. phaseoli* offspring much smaller than parents.
		Aye-le-zai	—	—	—	
		Pe-taing-taung	—	N	N	
		Thon-la-pe	—	—	—	
Pigeon-pea or red gram.	*Cajanus indicus*	Madras 17	N	—	N	*B. chinensis* occurred on it in the field.
		Do. A.	—		—	
		Madras 16	N	—	N	No eggs laid by *chinensis* which however occurred on it in the field.

APPENDIX—*contd.*

Pulses			Beetles			Remarks
Common English name	Scientific name	Burmese name or other description	*B. chinensis*	*B. analis*	*B. phaseoli*	
Pigeon-pea or red gram	*Cajanus indicus*	Madras 16 A . .	—	N	N	
		Do. 9 . .	—	—	—	*B. chinensis* occurred on it in the field.
		Do. 9 A . .	—	—	N	
		Do. 80 . .	—	N	—	Ditto.
		Do. 8 . .	—	—	—	Ditto.
		Do. 8 A . .	—	—	—	
		Do. 106 . .	N	—	—	Eggs laid by *chinensis* which also occurred on it in the field.
		Do. 106 A . .	—	N	—	
		Do. 102 . .	—	N	—	*B. chinensis* occurred on it in the field.
		Do. Hill variety A	—	N	N	Ditto.
		Ditto B	—	N	N	
		Ditto E	—	N	—	Ditto.
		Upper Assam No. 4	—	N	—	
		Do. No. 4 A.	—	N	—	
		Do. No. 4 B.	—	—	—	
		Do. No. 2 .	—	N	—	Ditto.
		Do. No. 1 .	—	N	—	Ditto,
		Do. No. 9 .	—	N	N	Ditto.
		Do. No. 9 A.	—	—	—	
		Do. No. 42 .	—	N	—	
		Do. No. 42 A	—	—	N	
		Do. No. 48 .	N	—	N	11 eggs laid by 2 pairs *chinensis.*
		Do. No. 48 A	N	—	—	No eggs laid by 2 pairs *chinensis.*
		Sylhet Brown .	N	N	N	No eggs laid by any of the three species. There were 1 pair *analis*, 2 pairs *chinensis* and 2 pairs *phaseoli*. *Chinensis* however occurred on it in the field.
		Sylhet White .	—	—	—	
		Sylhet Crimson .	—	—	—	*B. chinensis* occurred on it in the field.
		Gauhati No. 12 .	N	—	N	*B. chinensis* occurred on it in the field.
		Ditto A .	—	—	—	
		Ditto B .	—	—	—	
		Nagpur T 26 . .	—	N	—	

APPENDIX—*concld.*

Pulses			Beetles			Remarks
Common English name	Scientific name	Burmese name or other description	*B. chinensis*	*B. analis*	*B. phaseoli*	
Pigeon-pea or red gram	*Cajanus indicus*	Nagpur T 2 . .	—	N	—	*B. chinensis* and *albocallosus* occurred on it in the field.
		Do. E. B's 3 .	—	—	—	*B. chinensis* occurred on it in the field.
		Do. T 23 . .	—	—	—	
		Dacca No. 37 .	—	—	—	
		Do. No. 38 .	—	—	—	
		Do. No. 39 .	—	N	—	
		Pusa T 64 . .	—	—	—	
		Do. T 51 . .	—	—	N	
		Do. T 80 . .	—	—	—	
		Do. T 24 . .	—	—	N	
		Do. T 16 . .	—	—	—	
		Do. T 82 . .	—	—	—	
		Do. T 15 . .	N	—	N	No eggs laid by 2 pairs *chinensis* and 2 pairs *phaseoli*.
		Local from Hlwazin	—	N	—	*B. chinensis* occurred on it in the field. This and the following types collected in Burma.
		Local from Paukkoung.	—	—	N	
		Local from North Magwe.	—	—	N	
		New Era from Prome	—	—	—	
		New Era from Allanmyo	N	N	—	2 eggs only by 2 pairs *chinensis* which however occurred on it in the field. 12 eggs by 2 pairs *analis*.
		New Era from Paukkoung.	N	N	—	No eggs by 2 pairs *chinensis*.
		Do. with pods with dark streaks.	—	N	—	*B. chinensis* occurred on it in the field.
		Local type from Prome.	N	—	N	No eggs by 2 pairs *chinensis* which occurred on it in the field.
		Do. A .	—	N	N	
		A white type from meiktila	N	N	—	No eggs laid by 2 pairs *chinensis* which however occurred on it in the field. *B. albocallosus* also occurred on it in the field.
		Mandalay E. B's Pusa 26 .	—	—	—	
		Do. Pusa 34 .	—	—	—	*B. chinensis* occurred on them in the field.

STUDIES IN *FUSARIUM* WILT OF SANN-HEMP*

I. THE PHYSIOLOGY AND BIOLOGY OF *FUSARIUM VASINFECTUM* ATK.

BY

B. N. UPPAL

Plant Pathologist to the Bombay Government

AND

N. T. KULKARNI

Late Sann Investigator, Bombay Department of Agriculture

(Received for publication on 23rd March 1937)

(With one text-figure)

INTRODUCTION

SANN-HEMP, *Crotalaria juncea* L., which is extensively cultivated in many tropical countries, plays an important part in the agricultural economy of the Bombay-Deccan Canals. It supplies a cheap but valuable organic manure and is grown in rotation with such crops as sugarcane, *jowar* and wheat. Its value as a green manure is so firmly established in agricultural practice that sann-hemp, cane and cotton form a normal rotation in the Standard Manjri Method of sugarcane cultivation. Under conditions of normal growth sann-hemp yields about 30,000 lbs. of green dressing per acre, which supplies at less than half the cost almost as much dry matter as thirty cart-loads of farmyard manure. In addition to its value as a green manure crop, sann-hemp is grown on the Deccan Canals for renovating waste lands, and its cultivation is also encouraged on good lands to maintain the fertility of the soil and to prevent damage to these lands due to heavy irrigation.

Unfortunately, a destructive wilt disease of sann-hemp has broken out on the Deccan Canals and has caused heavy reduction in the yield of green dressing per acre. This is a matter of serious concern since under ordinary conditions farmyard manure is available only in limited quantities as stock-raising is not commonly practised on the Canals. An investigation was therefore undertaken with the object of making a comprehensive study of the disease and of obtaining a type of sann-hemp which would be resistant to wilt. In the following pages a short account of the disease and its causative organism is given. The results on the isolation of a wilt-immune type of sann-hemp and on the inheritance of resistance will form the subject of separate communications.

---

*The writers are indebted to the Trustees of the Sir Sassoon David Trust Fund for financial aid in this work.

DISTRIBUTION AND ECONOMIC IMPORTANCE

The earliest reference to wilt in Bombay is by Chibber [1914], but wilt is now the commonest disease of sann-hemp in this Presidency. It has also been reported by Mitra [1934] from Pusa in Bihar. In other countries Vincens [1921] has reported this wilt from French Indo-China, Briant and Martyn [1929] from Trinidad, and Martyn [1933] from British Guiana. Wilt has also been recorded on other species of *Crotalaria* such as *C. striata* in Uganda [Hansford, 1932] and *C. usaramoensis* in Dutch East Indies [Van Hall, 1925] and in French Indo-China [Vincens, 1921]. The disease seems to have a wide geographical distribution and is common in tropical lands where sann-hemp and allied species of *Crotalaria* are cultivated.

Wilt in sann-hemp is very serious in Bombay, although the damage varies according to the season and locality. On the Manjri Sugarcane Station near Poona the disease had reached such serious proportions that in 1929 the quantity of green dressing per acre was reduced to 17,000 lbs., even to 1,000 lbs. in some very badly infected plots, as against 30,000 lbs. of green matter obtained under conditions of normal growth. A count made in 1930 of diseased plants in sann-hemp plots on the Manjri Station showed that mortality from *Fusarium* wilt was as high as seventy per cent in some plots. The condition at Manjri is generally representative of many sugarcane farms on the Deccan Canals where successive cultivation of sann-hemp has greatly intensified the existing infection and has rendered the land unsuitable for profitable growing of sann-hemp as a green manure crop.

As a conservative estimate, the damage due to *Fusarium* wilt on the Deccan Canals may run up to twenty-five to thirty per cent. Recently Mitra [1934] has estimated the loss at twenty-three to sixty per cent in the cultivators' fields around Pusa. Briant and Martyn [1929] consider wilt as a serious disease in Trinidad, especially in areas where sann-hemp is grown for seed production.

Wilt is thus a serious menace to the Manjri Standard Method of sugarcane cultivation on the Deccan Canals, in which sann-hemp plays as important part as green manure. Various attempts have been made to find a substitute for sann-hemp as a green manure crop, but these efforts have met with little success so that sann-hemp is still the only source of cheap organic manure for sugarcane.

SYMPTOMS

The symptoms of sann-hemp wilt have been described by Briant and Martyn [1929], Wright and Leach [1931] and Mitra [1934]; but these will be supplemented here briefly.

Under field conditions yellowing of leaves is the first visible symptom and starts with the oldest leaves at the base and gradually proceeds upwards. The chlorosis may invade the entire leaf or a portion of it near the petiole or at the tip. This is followed by the drooping or drying of chlorotic leaves, resulting ultimately in the death of the plant. Unilateral chlorosis of leaves has been observed, although this is not a common symptom. Concurrent with the yellowing of leaves, the petiole reveals a brownish discoloration of the vascular tissue, which is one of the symptoms for recognising a diseased plant in the field.

Plants may be infected at any stage of their growth, although wilting is not much noticeable until environmental conditions are favourable. If infection occurs early, young seedlings may die in a few hours after emergence from the soil. When older plants are affected, wilting occurs slowly and these plants collapse in two to four weeks. Yellowing of leaves is very marked in plants that wilt slowly. Wilting is very rapid in September and October, but plants wilt gradually early in the season in June and July. If the stalks of diseased plants are split open longitudinally, they are found to have discoloured bundles. This discoloration may extend from the roots into the stem and petiole and in some cases has been traced up to the hilum of the green seed in the pod. On pulling up diseased plants some dead roots may be found, whilst others are apparently healthy. After the death of a plant the fungus grows into the parenchymatous tissue of the stem and comes out on its surface as pink masses of sporodochia ; sclerotial bodies may be found at the collar of the stem.

MORPHOLOGICAL AND CULTURAL CHARACTERS

The cultural studies were made in order to obtain the complete range of spore forms of *Fusarium vasinfectum* isolated from wilted sann-hemp plants. For the production of normal types it is essential to use different media, since Wollenweber *et al.* [1925] and Reinking and Wollenweber [1927] have pointed out that differences may arise in the same species when normal spores are produced under various conditions. The cultures on various media were grown at room temperature ranging from 23 to 27°C., with an average of about 25°C. Ridgway's [1912] colour nomenclature has been followed in describing colour production in the media.

Growth on various media

Richards' agar.—Mycelium vinaceous pink in the centre of the plate and cream colour along the sides. Growth sub-aerial and slightly raised at the centre. Cultures (when viewed from above) orient pink at the centre and water green all round. Pinnotes extensively developed and cream colour. Macroconidia produced in abundance, rich in contents, sickle-shaped, mostly 3-septate but septation not well marked in spores in young pinnotes. Microconidia few. When spores are produced in abundance, the culture is orange pink.

Potato agar, 5 per cent dextrose.—Cultures twenty days old have no aerial mycelium and are yellowish glaucous. Pinnotal spore masses extensively developed, flesh colour at the top and salmon colour at the base of the culture. Macroconidia abundant, typically 3-septate. Microconidia few. Blue sclerotial bodies may be formed.

Potato hard agar.—Cultures twenty days old have a medium dense and matted, buff pink mycelium. Pinnotes extensive, flesh colour in the centre of the plate and pale olive buff along the sides (when viewed from above), and pale salmon (when viewed from below). Macroconidia and microconidia abundant.

Potato-tuber plug.—Cultures about one month old have a dense matted, pink, aerial mycelium. Macroconidia few; microconidia numerous. Chlamydospores and sclerotia absent.

Oat-meal agar.—Cultures about one month old are characterised by a scant, aerial mycelium. The substratum is coloured dark vinaceous. Macroconidia few; microconidia numerous.

Brown's agar.—Cultures about one month old have a scant, submerged mycelium. Pinnotes are developed. Macroconidia and microconidia in about equal proportion.

Rice.—Cultures about one month old are characterised by a dense, sub-aerial growth, flesh ochre when seen from the top and carnelian red at the base. A strong benzolic odour is produced.

Measurements of conidia on different media

Potato hard agar; culture twenty-five days old; conidia from pinnotes:

Conidia—

- 0-septate, one per cent, 21·5 × 2·5 (20-22·5 × 2·5) μ.
- 1-septate.
- 2-septate, 0·5 per cent, 22·5 × 4 μ.
- 3-septate, 84 per cent, 33·2 × 4 (22·5-47·5 × 2·5-5) μ.
- 4-septate, 13·5 per cent, 44·5 × 4 (37·5-55 × 3-5) μ.
- 5-septate, one per cent, 57·5 × 5 (50-60 × 5) μ.

Rice; culture twenty days old; conidia from mycelium and pinnotes:

Conidia—

- 0-septate, 2·5 per cent, 29 × 3·2 (22·5-35 × 2·5-4) μ.
- 1-septate, 12 per cent, 26·9 4 (20-32·5 × 2·5-5) μ.
- 2-septate, 4 per cent, 26·2 × 3·4 (20-35 ×2·5-5) μ.
- 3-septate, 74 per cent, 32·2 × 3·9 (22·5-45 × 2·5-5) μ.
- 4-septate, 5 per cent, 41 × 4·2 (35-50 × 4-5) μ.
- 5-septate, 2·5 per cent, 44·6 × 4·6 (35-52·5 × 4-5) μ.

Oat-meal agar; culture twenty days old; conidia from mycelium and pseudopinnotes:

Conidia—

- 0-septate.
- 1-septate, one per cent, 28·7 × 3·5 (27·5-30 × 3-4) μ.
- 2-septate, one per cent, 25 × 3 (22·5-27·5 × 3) μ.
- 3-septate, 93 per cent, 36·5 × 4·3 (25-50 × 2·5-5) μ.
- 4-septate, 4 per cent, 48 × 4·5 (40-60 × 3-5) μ.
- 5-septate, one per cent, 48·7 × 5 (47·5-50 × 5) μ.

Wilted sann-hemp stems; conidia from natural sporodochia:

Conidia—

- 0-septate, 2 per cent, 37·5 × 3·5 (32·5-47·5 × 3-4) μ.
- 1-septate, 2 per cent, 26·2 × 3·2 (20-37·5 × 3-4) μ.
- 2-septate.
- 3-septate, 87 per cent, 34·2 × 4 (25-47·5 × 2·5-5) μ.
- 4-septate, 7·5 per cent, 43·2 × 4·3 (32·5-52·5 × 4-5) μ.
- 5-septate, 1·5 per cent, 46·2 × 4·6 (45-50 × 4-5) μ.

Average of the above measurements

Conidia—

- 3-septate, 85 per cent, 33 × 4 (22·5-50 × 2·5-5) μ.
- 4-septate, 7·8 per cent, 44 × 4·1 (32·5-60 × 3-5) μ.

Conidia are from 0- to 5-septate, mostly 3-septate; 33 × 3·9 μ in size (average); typically sickle-shaped, base pedicellate; hyaline but salmon pink in mass. On rice medium the colour produced is characteristic of the section Elegans.

PATHOGENICITY

Numerous isolations have been made from wilted sann-hemp plants collected in widely separated localities. In all cases a fungus has been obtained, which is indistinguishable from *Fusarium vasinfectum* Atk. commonly isolated from wilted cotton plants.

The first inoculation experiments were made in the monsoon of 1930. Pure cultures of sann-hemp *Fusarium* were multiplied on sann-meal-sand medium prepared by mixing equal quantities of sann-hemp meal and quarts sand. Soil was sterilised in an autoclave at twenty pounds pressure for two hours on two successive days and inoculated by stirring cultures of *Fusarium* into it. This inoculated soil was filled in small pots previously washed in a strong solution of copper sulphate. After ten days sann-hemp seeds, which had been disinfected in a one in 1000 solution of mercuric bichloride for ten minutes, were sown in each pot. An equal number of pots of sterilised, uninoculated soil was planted as control. Two weeks after the emergence of seedlings a number of plants in pots of inoculated soil showed the characteristic yellowing of leaves; thereafter the disease progressed rapidly and by the end of a month all chlorotic plants had wilted. The wilted plants were plated out on potato-dextrose agar in all cases, and the typical *Fusarium* was found to be present. No wilt appeared in any of the control pots. The results of this experiment are given in Table I.

TABLE I

Results of inoculations with pure cultures of Fusarium vasinfectum *isolated from wilted sann-hemp plants*

1930			1931			1932		
Regional source of strain	No. of plants	Per cent of wilt	Regional source of strain	No. of plants	Per cent of wilt	Source of strain	No. of plants	Per cent of wilt
Manjri	37	73	Manjri	11	100	Bombay strain	20	100
Kopargaon	10	70	Kopargaon	5	100	Trinidad strain	12	100
Kirkee	20	100	Gokak	5	80	Natural sporodochia (Bombay strain)	20	90
Gokak	10	80	Vason	10	80			
			Jalalpur	3	100			
			Ponia	10	80			
			Dongri	8	88			
			Amalsad	20	90			
			Rentlav	7	89			
			Kacholi	10	80			
			Sukhlav	10	100			
			Hari	10	80			
			Motiwada	10	100			

In 1931 fresh cultures of *Fusarium* were made from wilted sann-hemp plants collected in widely separated localities in the Bombay Presidency and were multiplied, as usual, on sann-meal-sand medium. These cultures were tested for their pathogenicity in the same manner as in the previous year. All isolations proved pathogenic on sann-hemp and did not vary much in virulence. Re-isolations were made in each case and resembled the original cultures. Adequate controls were included in this series and in all cases these plants remained healthy. These results are also summarised in Table I.

In 1932 the above experiment was repeated using three pots of sterilised soil. The treatment of the soil in these pots was as follows : One pot was inoculated with a culture of the Bombay strain isolated from wilted plants at Manjri, the other with a culture of the strain received from Trinidad, and the third with the scrapings of sporodochia of the fungus on the dead stem of a wilted sann-hemp plant. The results in Table I show that there was no variation in the virulence of the strains of the fungus obtained locally and from Trinidad. In all cases control plants remained healthy.

RELATION OF TEMPERATURE TO FUNGUS GROWTH

The growth of *Fusarium vasinfectum* at various temperatures was studied in plates containing 20 c. c. of Richards' agar. A bit of the mycelium from a rapidly growing culture of the fungus was transferred to the centre of each plate, which was then incubated at the desired temperature. Duplicate plates were carried at each temperature, and colony diameters were measured at the end of the sixth day. Table II gives the average of the radial growth at each temperature.

TABLE II

Growth of Fusarium vasinfectum *at various temperatures*

Temperature °C.	Colony diameter in cm. after 6 days
4·4	0·0
7·2	0·4
11·2	1·1
18·5	3·6
20·0	4·0
25·0	5·8
30·0	5·8
32·0	3·2
34·0	3·0
36·0	0·5
40·0	0·0

Fusarium vasinfectum has a wide growth temperature range, the minimum and maximum temperature of growth being 7·2° and 36°C., respectively. The fungus grows most rapidly at 25° to 30°C. This is in agreement with the results of Mitra and Kheswalla [1935] for the cotton strain of the fungus, although Kulkarni [1934] has reported 26° to 28°C. as the optimum temperature of the latter.

RELATION OF SOIL TEMPERATURE TO WILT

It is generally recognised that soil temperature markedly influences the development of vascular *Fusarium* diseases. Gilman [1916], Tisdale [1923] and Tims [1926] have studied the relation of soil temperature to the development of yellows in cabbage caused by *Fusarium conglutinans,* and have come to the conclusion that cabbage yellows is most destructive at soil temperatures ranging from 26° to 29°C. These investigators also observed a close correlation between the vegetative growth of the fungus in culture at different temperatures and the development of yellows at corresponding soil temperatures. Clayton [1923, 1] also found that tomato wilt caused by *Fusarium lycopersici* reaches its maximum development at a soil temperature of about 28°C., which is also the optimum temperature for the vegetative growth of the parasite in culture. Tisdale [1917] has likewise studied the effect of soil temperature on wilt in flax caused by *Fusarium lini,* and has found its temperature relations to be identical with those of tomato wilt and cabbage yellows.

These results on the relation of soil temperature to *Fusarium* wilts are in accord with the general idea that temperature affects the development of these diseases through its direct influence on the parasites. The only experiments not in accord with this conclusion are those of Kulkarni [1934] on cotton wilt at Dharwar. Kulkarni found that *Fusarium vasinfectum* is an extremely aggressive parasite in a range of soil temperatures of 20° to 25°C., whilst the optimum range for the vegetative growth of the fungus is about 26° to 28°C., thus indicating a negative correlation between the optimum temperature for growth of the parasite and that for the development of wilt in cotton. He therefore concluded that soil temperature influences cotton wilt primarily through its effect on the host.

Recently Mundkur [1935] has brought forth evidence, based entirely on field data, that " in sann-hemp the incidence of wilt is favoured by soil temperatures ranging approximately from 28° to 33°C." and has further expressed the view that the optimum temperature range for sann-hemp wilt is higher than that considered by Jones *et al.* [1926] to favour the development of *Fusarium* wilts in general. Since a satisfactory analysis of this important contributing factor is of special interest, particularly in the isolation of wilt-resistant types of sann-hemp, an investigation was made of soil temperature as it affects the development of sann-hemp wilt.

Gross cultures of the sann-hemp wilt fungus were multiplied on equal weights of sann-meal-sand medium in giant Erlenmeyer flasks and incorporated in aliquots of sterilised soil under identical conditions. The inoculated soil was frequently stirred up and kept moist to permit of the rapid development of vegetative mycelium. Ten days later the soil was filled in galvanised iron cans, which were then removed to soil-temperature tanks of Wisconsin type. Subsequently, sann-hemp seed, previously surface-disinfected in a solution of one in 1000 mercuric bichloride for ten minutes, was sown in the cans. Measured quantities of water were added to each can twice a week and, after the emergence of seedlings, soil was gently stirred up occasionally up to a depth of about two inches. In this way sann-hemp plants were maintained in a thrifty condition of growth. Re-isolations were made from wilted plants in all cases, and at the conclusion of each experiment, usually forty days after sowing, the surviving plants were either examined for discoloration of vascular bundles or plated out on potato-dextrose agar. Three experiments will be briefly described.

In the first experiment, the results of which are given in Table III, five soil-temperature tanks were set up at 20°, 25°, 30°, 35° and 40°C., but owing to the prevalence of very cool weather in December there were fluctuations in temperatures of these tanks ; the figures in the table are therefore mean soil temperatures obtained during the period of test. Wilt developed in sann-hemp at all temperatures tested. It appeared after twelve days in mean soil temperatures of 29·4°, 32·4° and 37°C., but was progressively late in appearing at lower levels Wilt appeared after fifteen days at 26·8° and after seventeen days at 21·4°C.

TABLE III

Effect of soil temperature on the development of wilt in sann-hemp

	Soil temper-ature* °C.	Total No. of plants	After 12 days		After 20 days		After 30 days		After 40 days	
			Per cent of in-fection	Per cent of wilt	Per cent of in-fection	Per cent of wilt	Per cent of in-fection	Per cent of wilt	Per cent of in-fection	Per cent of wilt
Experiment I	21·4	56	..	1·8	..	16·0	..	57·0	..	..
	26·8	78	..	14·0	..	56·4	..	84·6	..	..
	29·4	62	..	12·9	..	61·0	..	75·8	..	..
	32·7	71	..	5·6	..	28·0	..	42·0	..	..
	37·0	61	..	14·7	..	23·0	..	23·0	..	..
Experiment II	18·0	81	1·2	1·2	2·4	2·4	2·4	2·4	34·5	2·4
	25·6	70	0·0	0·0	3·0	3·0	18·0	6·0	52·8	37·0
	27·6	75	1·3	1·3	1·3	1·3	13·0	4·0	57·3	39·0
	33·5	85	2·3	2·3	2·3	2·3	7·0	3·5	10·5	7·0
	37·0	45	8·8	8·8	8·8	8·8	8·8	8·8	8·8	8·8
Experiment III	24	56	3·5	0·0	35·7	5·3	45·0	9·0	51·7	28·5
	26	61	19·6	3·2	39·3	18·0	64·0	49·0	85·2	65·5
	28	66	15·3	4·5	36·5	25·7	84·8	70·0	84·8	70·0
	30	64	14·0	7·9	54·0	23·4	85·9	39·9	85·9	59·3
	32	66	35·0	3·0	35·0	10·0	40·0	20·0	56·0	45·4

*In the first and second experiments figures under ‘soil temperature’ are mean soil temperatures.

The second experiment was also done in the cold season when it was somewhat difficult to control temperatures in tanks owing to a sharp fall of temperature during the night. The figures in the table are therefore mean soil temperatures during a period of forty days. The results are in general agreement with those of the first experiment, although the number of plants that wilted after forty days was comparatively smaller owing to unfavourable air temperatures.

The third experiment was started on 25th June and concluded in the first week of August. During this period air temperatures were very favourable to the development of wilt, and no difficulty was experienced in maintaining the desired temperatures in soil-temperature tanks, since the fluctuations, whenever occurred, were always within a degree in either direction. The soil used in previous tests was replanted with disinfected sann-hemp seed and held at 24°, 26°, 28°, 30° and 32°C. At 24° the disease appeared after seventeen days and progressed slowly. At 26° to 30° it was characterised by earliness of attack as well as severity of infection, since the period of incubation of the disease was ten days and plants wilted rapidly. At 32° infection again became less severe accompanied by an increase to seventeen days in the incubation period.

It will be seen from these experiments that sann-hemp wilt caused by *Fusarium vasinfectum* reaches its maximum development at soil temperatures of 26° to 30°C. This temperature relation of the disease shows a remarkably close parallelism with that of the parasite, which makes the best vegetative growth at 25° to 30°C. as measured by colony diameter ; at higher levels the radial growth of the fungus falls off more sharply than the incidence of wilt (Fig. 1). As in other *Fusarium* diseases of this group, soil temperature therefore influences sann-hemp wilt primarily through its effect on the parasite, although, as previously noted, the work of Kulkarni [1934] on cotton wilt caused by *Fusarium vasinfectum*, to which one of us will revert in another publication, is an exception to this general conclusion. This evidence also does not support the conclusion of Mundkur [1935] that sann-hemp wilt has a higher temperature optimum than the allied vascular *Fusarium* diseases such as cabbage yellows, tomato wilt or flax wilt. In all these cases the curve of disease development is characterised by a gradual rise to its optimum, which lies between 25° and 30°C., and then falls off sharply as the temperature rises above this level.

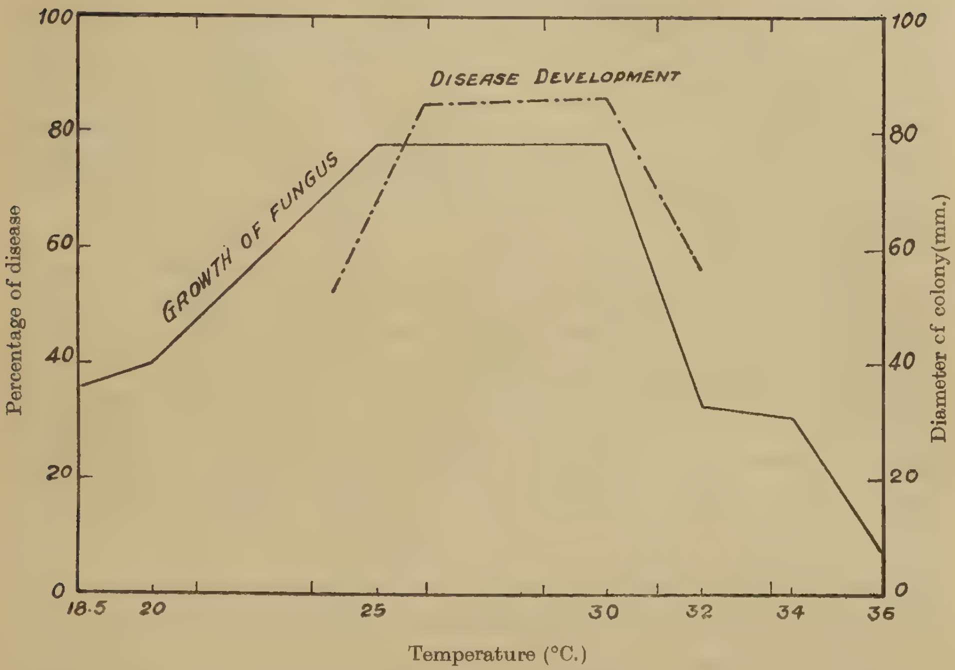


FIG. 1. Relation between growth of *Fusarium vasinfectum* and development of disease in sann-hemp at various temperatures.

Although the relation of soil temperature to the normal development of sann-hemp has not been experimentally studied, yet there are enough indications from the general experiments that sann-hemp grows luxuriantly at about 25° to 35°C. ; at higher and lower levels plants become stunted and flower prematurely. This observation is also in accord with the general experience that sann-hemp makes stunted growth and produces little seed when grown in the cold or hot season under irrigation. Thus, whilst sann-hemp will grow normally up to about 35°C., sann-hemp wilt progressively declines in severity above 30°C. Temperatures lower than 25°C. are not favourable either to the normal development of sann-hemp or to vigorous infection.

RELATION OF SOIL MOISTURE TO WILT

Besides soil temperature, soil moisture also operates as a limiting factor in the development of vascular *Fusarium* diseases. Clayton [1923, 2] has shown that soil moistures suitable for vigorous growth of the host had no effect on tomato wilt; but dry and moisture-saturated soils which adversely affected the host also checked the disease proportionally. Ryker [1935] has also come to a similar conclusion that *Fusarium* yellows of celery is checked in low moistures when the host development is much retarded. Kulkarni [1934], on the other hand, found that soil moisture had no effect on the development of cotton wilt. As regards sann-hemp wilt, Wright and Leach [1931] concluded from field observations that a high moisture content of the soil was without effect on the incidence of the disease. The following experiment was done to study the effect of soil moisture in correlation with optimum soil temperature on the development of sann-hemp wilt.

The method of preparation of inoculum and its incorporation into steam-sterilised river soil was essentially the same as described previously. The inoculated soil was filled in galvanised iron cans, and water was introduced through a long glass tube leading from the surface to an inverted flower pot buried in the soil. The moisture content of the soil in each can was made up on the oven-dry basis. After the seedlings emerged, saw-dust was spread over the surface in a thick layer to prevent loss of water by evaporation from the cans, and soil-moisture content was kept constant by weighing every day and restoring the original weight by the addition of water. With dry soils, no great difficulty was experienced in keeping the moisture content constant, and only small quantities of water were required to be added every day. The rate of loss of water from soils with high moisture content was also not so high as to cause wide fluctuations.

Disinfected sann-hemp seed was sown in the cans which were then removed to soil-temperature tanks held at 26° to 28° C. For a period of five days from the date of sowing, sufficient water was added to each can to allow germination and establishment of seedlings to proceed normally, and subsequently the cans were adjusted to their respective gross weights which had previously been determined for each percentage of soil moisture. Adequate controls of non-inoculated soil were provided for each soil moisture tested. The experiment was run for forty days.

The results in Table IV indicate that sann-hemp wilt is extremely virulent in fifteen per cent soil moisture, although the number of diseased plants in twenty per cent soil moisture was also almost as high as in fifteen per cent. The disease declined progressively as the moisture content of the soil was raised so much so that in forty per cent soil moisture the plants exhibited a high degree of resistance to wilt and almost sixty-five per cent of plants showed no symptoms of the disease. It was also observed that in low soil moistures the disease was characterised by earliness of attack, and the incubation period increased with rise in soil-moisture content. These results are contrary to those of Clayton [1923, 2] for tomato wilt, which develops at a slower rate in plants retarded by low soil moisture, but is destructive in medium soil moistures in which tomato plants make a fairly vigorous growth.

TABLE IV

Effect of soil moisture on the development of wilt in sann-hemp at 26° to 28°C.

Percentage of soil moisture	Total No. of plants	After 12 days		After 20 days		After 30 days		After 40 days	
		Per cent of infection	Per cent of wilt	Per cent of infection	Per cent of wilt	Per cent of infection	Per cent of wilt	Per cent of infection	Per cent of wilt
15	41	29·2	26·8	61·0	41·4	92·6	68·2	92·6	75·6
20	23	21·7	21·7	30·5	21·7	69·5	21·7	82·6	26·0
25	32	0·0	0·0	15·6	9·3	50·0	21·7	62·5	21·7
30	57	1·7	0·0	7·0	3·5	28·0	10·5	50·0	14·0
35	57	0·0	0·0	7·0	3·5	30·0	16·0	40·0	21·3
40	49	0·0	0·0	14·2	10·2	24·0	16·0	34·0	20·4

It was further observed that many apparently healthy plants growing in different soil-moisture contents showed discoloration of the root, which was confined to the subterranean part and did not extend up the stem. There was also no indication that root discoloration was more in evidence in certain soil moistures than in others. There were thus a number of plants growing in different soil moistures, which did not show any external symptom of wilt but were nevertheless diseased. Isolations from the roots of such plants gave pure cultures of *Fusarium vasinfectum*. Clayton [1923, 2] has observed similar root discoloration in apparently healthy tomato plants affected by wilt, but found that this discoloration was evident only in plants growing in dry soil and further was not confined to the subterranean part as is the case with sann-hemp wilt.

It is evident from the above discussion that sann-hemp wilt, like cabbage yellows, is very destructive to sann-hemp plants growing in soil of low moisture content and at temperature in the neighbourhood of 28°C. Since soil moisture does not vary widely under the normal range of field conditions during the monsoon, soil temperature generally operates as the limiting factor in determining the severity of sann-hemp wilt. These results are in accord with the common observation that sann-hemp plants wilt rapidly during the monsoon in dry periods following rains.

THE ENZYMES OF *Fusarium vasinfectum*

In the investigation of the principal enzymes of *Fusarium vasinfectum*, Crabill and Reed's [1915] method of testing for the enzymes in the mycelium was followed. For this study a stock medium of the following composition was made :

Magnesium sulphate	0·5 grm.
Di-potassium hydrogen phosphate	1 grm.
Potassium chloride	0·5 grm.
Ferrous sulphate	0·01 grm.
Agar	20 grms.
Water	1000 c.c.

To this stock medium carbon-containing compounds were added, and after sterilisation the media were poured in thin layers into plates, with constant shaking to secure uniform distribution of the compounds. The plates were inoculated with a small piece of inoculum from a young culture of *Fusarium vasinfectum* and incubated at room temperature (25°C.).

Diastase.—To 500 c.c. of the stock medium ten grms. of potato starch was added, the medium sterilised and poured in thin layers into plates, with constant shaking. The plates were inoculated at the centre and incubated at 25°C. The fungus made very poor growth and there was no 'halo' or clearing of the medium around the colony, indicating that starch had not been dissolved from the medium. Hence the mycelium of *Fusarium vasinfectum* does not excrete the enzyme diastase.

Inulase.—Inulin agar was prepared by adding five grms. of inulin to 1,000 c.c. of the stock medium. The medium was poured into plates, which were inoculated at the centre and incubated at 25°C.

The ability of the organism to dissolve inulin is tested by the amount of mycelial growth and spore production. *Fusarium vasinfectum* made very poor growth on inulin agar and produced a few deformed spores. This shows that the mycelium of the fungus does not contain the enzyme inulase.

Emulsin.—A one per cent solution of amygdalin was added to the stock medium. After sterilisation amygdalin agar was poured into plates, which were inoculated at the centre and incubated at 25°C. as usual.

If the fungus makes good growth on amygdalin agar, it is assumed that amygdalin is broken down by the organism and partly converted to glucose, which is easily assimilated. *Fusarium vasinfectum* made very good growth on amygdalin agar, showing that its mycelium contains the enzyme emulsin.

Lipase.—Litmus-cream agar was used to test the presence of lipase in the mycelium. Twenty grms. of agar was dissolved in 400 c.c. of water in an autoclave, and then poured into 50 c.c. of forty-eight per cent separator cream brought up to 600 c.c. with distilled water and sterilised separately in an Arnold steriliser. To this medium enough of sterile litmus solution was added to impart a deep blue tint. The medium was then poured into plates, which were inoculated and incubated as usual.

Fusarium vasinfectum made good growth on litmus-cream agar and produced a zone of sharp red colour in the plate. The red colour of the medium was due to the action of free fatty acids on litmus produced as a result of the splitting of fats by the action of lipase. Hence it is concluded that the mycelium of the fungus contains the enzyme lipase.

Erepsin.—Casein agar was prepared by adding one per cent of finely divided casein to the stock medium. The plates containing casein agar were inoculated at the centre and incubated at 25°C. *Fusarium vasinfectum* made good growth on casein agar, and there was a ‘ halo ’ produced in the medium under the colony indicating the digestion of casein by the fungus. This shows that the fungus mycelium excretes the enzyme erepsin.

Trypsin.—Egg-albumen agar was used for this purpose and was prepared by mixing the white of three eggs, well beaten in 75 c.c. of water, with 500 c.c. of the stock medium. The albumen was then coagulated by cooking the medium for half an hour in an autoclave.

Fusarium vasinfectum was grown on egg-albumen agar, and the cultures were incubated at 25°C. The fungus grew profusely on the medium and digested the coagulum, producing a halo of clear agar surrounding the colony. This indicates that the fungus mycelium contains the enzyme trypsin.

Amidase.—Asparagin-rosolic-acid agar was used and was prepared by adding to the stock medium 0·5 per cent of asparagin and 5 c.c. of two per cent rosolic acid per litre. This medium has a slight yellow tint.

Fusarium vasinfectum, when grown on this medium, produced a beautiful red reaction, indicating that asparagin was acted on by amidase liberating ammonia, which changed the yellow of rosolic acid to brilliant red. Hence it is concluded that there is a considerable quantity of amidase in the mycelium of the fungus.

Cytase.—Kellerman's method of preparing the medium for demonstrating the presence of cytase in the mycelium, as described by Crabill and Reed [1915], was followed. The plates were inoculated and incubated as usual. The fungus failed to grow on the medium, showing that its mycelium does not contain the enzyme cytase.

PRODUCTION OF TOXIC SUBSTANCES

Many theories have been advanced to explain wilting in plants induced by vascular parasites. One of these theories assumes the production of toxic excretory substances by the invading organism, and wilting is believed to be caused by the action of these substances. Rosen [1926] has suggested that an inorganic salt in the form of nitrite is one of the toxic ingredients inducing wilt in cotton. Wright and Leach [1931], however, were unable to detect the presence of nitrite in the filtrate of *Fusarium vasinfectum* causing wilt of sann-hemp in Trinidad, which was further found to have no toxic effect on the plants. The following experiments were done to determine whether nitrite is produced by the action of *Fusarium vasinfectum* in the presence of nitrogen in the culture media, and whether this nitrite is toxic to sann-hemp plants.

Experiment I.—Rosen [1926] has shown that the cotton wilt fungus produces nitrite from inorganic nitrate but not in the presence of organic nitrogen. Wright and Leach [1931] grew the sann-hemp *Fusarium* in a solution of the following composition, to which 1·3 grm. of sodium nitrate or one grm. of sodium asparaginate was added as source of inorganic and organic nitrogen, respectively. They tested for nitrite in the culture filtrate by means of safranin-T and hydrochloric acid, but were unable to detect its presence.

Calicum sulphate	0·84 grm.
Magnesium sulphate	0·25 grm.
Potassium dihydrogen phosphate	0·25 grm.
Ferric chloride	trace.
Glucose	20 grms.
Water	1,000 c.c.

The above solution of Wright and Leach was prepared in a large quantity, and to half of it was added sodium nitrate and to the other half sodium asparaginate was added. Each of the two solutions was distributed in 100 c. c. volumes in ten flasks and then sterilised in an autoclave. When cold the flasks were inoculated with the mycelium of the sann-hemp *Fusarium* grown on potato-dextrose agar, and incubated at 25°C. After intervals of two, four, seven, fourteen and thirty-one days, the culture solution from two flasks in each series was passed separately through filter paper, and the filtrates obtained were tested for the presence or absence of nitrite. Using sulphanilic acid-*a*-naphthylamine method as described in " Manual of Methods for Pure Culture Study of Bacteria " published by the Society of American Bacteriologists, a distinct pink colour indicating the presence of nitrites was produced in the filtrates of solutions provided with inorganic nitrogen and in which the fungus had grown ; whilst similar filtrates of the synthetic medium to which organic nitrogen as sodium asparaginate was added all gave negative tests.

The effect of the filtrates on the germination of sann-hemp seed was tested and it was found that only the filtrates derived from the medium containing inorganic nitrogen exerted a slight inhibitory action on germination.

Experiment II.—The object of this experiment was to determine whether the fungus can produce nitrite in Richards' solution. The fungus was grown in 100 c.c. portions of the solution in Erlenmeyer flasks, and at the end of six, eight, ten, fourteen and thirty-one days filtrates of the fungus cultures were obtained by the usual method. A very positive test for nitrites was obtained in all the filtrates by means of sulphanilic acid-*a*-naphthylamine.

To test the effect on seed germination, filter papers were moistened with filtrates of ten and twenty-four-day-old cultures of the fungus in Richards' solution and then placed in sterilised Petri dishes. Surface-sterilised sann-hemp seed was spread out to germinate on these filter papers. It was observed that a marked inhibitory effect was produced on germination by the ten-day-old filtrate, reducing the germination of seed to thirty-five per cent ; whilst germination was almost completely inhibited by the filtrate of the culture medium in which the fungus had grown for twenty-four days.

Experiment III.—In view of the conflicting evidence on the production of nitrite from inorganic nitrate by *Fusarium vasinfectum*, cultures of the sann-hemp wilt fungus were grown in Richards' solution for ten, twenty-four and twenty-eight days. At the end of these periods determinations* for the production of nitrite nitrogen were made, and these figures are shown in Table V and are compared with the data given by Rosen [1926] and Kulkarni and Mundkur [1928] for the cotton wilt fungus.

---

*The determinations of nitrites in culture filtrates were made by Dr. V. N. Gokhale and Mr. D. M. Ranade, Agricultural Chemistry Section, College of Agriculture, Poona.

TABLE V

Amount of nitrite nitrogen in filtrates of Fusarium vasinfectum

Name of worker	Age of filtrate	Nitrite nitrogen in mg. per c. c. of filtrate	
		F. vasinfectum from sann-hemp	*F. vasinfectum* from cotton
Rosen . .	2-3 weeks . .	....	0·0125 to 0·04
Kulkarni and Mundkur .	Not mentioned .	....	0·000025
Present writers .	10 days . .	0·00011	
	24 days. . .	0·0015	
	28 days. . .	0·0022	

It will be seen from Table V that appreciable quantities of nitrite nitrogen were produced by the sann-hemp wilt fungus in Richards' solution at the end of twenty-four and twenty-eight days' growth. These results, while agreeing somewhat with the figures given by Rosen [1926] for *Fusarium vasinfectum* from cotton, are contrary to those of Kulkarni and Mundkur [1928] who were unable to detect appreciable quantities of nitrite in culture filtrates.

Experiment IV.—This experiment was done to test whether spores of *Fusarium vasinfectum* can reduce nitrates to nitrites. The spores of the fungus growing on potato-dextrose agar were scraped carefully from pinnotes with a clean, dry slide and suspended in distilled water. Two c.c. of the spore suspension, when added to 48 c.c. of a solution of sodium nitrate, gave a suspension containing one per cent sodium nitrate. Ten c.c. portions of this spore suspension were distributed in test tubes and tested for nitrites after three, five, seven, twelve, thirteen and sixteen days by the usual method. In all cases a positive test for nitrites was obtained ; the control containing one per cent sodium nitrate without spores gave a negative test.

A similar test in which spores of the fungus were suspended in one per cent sodium asparaginate for one, two, eight and fourteen days gave negative results, thus indicating that nitrite is not produced by the action of the fungus spores in the presence of organic nitrogen.

Experiment V.—Wright and Leach [1931] have stated that in the filtrate of the wilt fungus sann-hemp plants remained turgid even after forty-eight hours. In order to test this point, the culture solution containing sodium nitrate was prepared according to the formula of Wright and Leach, and the sann-hemp wilt fungus was grown in equal portions of it in Erlenmeyer flasks at 25°C. for fourteen and seventeen days. Filtrates were obtained in the usual manner and distributed in equal volumes in test tubes. Young seedlings about a fortnight old were placed in these tubes. The original solution was used as control.

Filtrate	After 24 hours	48 hours	68 hours	102 hours
14-day old .	Cotyledons drooping	True leaves still turgid	Drooping	Wilted
17-day old .	Turgid	Drooping	Wilted	
Control . .	Turgid	Turgid	Turgid	Turgid

It will be seen that in the fourteen-day-old filtrate sann-hemp plants were still turgid after forty-eight hours, but were all dead after 102 hours. The seventeen-day-old filtrate, however, was more toxic to the plants, and their leaves began drooping after forty-eight hours. These results in part agree with those of Wright and Leach [1931], but are contrary to their observation that the sann-hemp wilt fungus produces no poisonous substances in the culture medium toxic to the plants.

In another experiment the sann-hemp wilt fungus was grown in Richards' solution for about five months. The filtrate obtained was divided into two portions : one was boiled for a few minutes and the other was left unboiled. Each portion was distributed in equal volumes in five test tubes, and a young seedling was placed in each tube. The original Richards' solution served as control.

Filtrate	After 14 hours	20 hours	24 hours
Unboiled . .	Drooping	Drooping	Wilted
Boiled . .	Drooping	Drooping	Wilted
Control . .	Turgid	Turgid	Turgid

Both the boiled and unboiled filtrates produced rapid drooping of leaves followed by death in twenty-four hours. Sann-hemp plants in both the filtrates wilted in a similar manner, the leaves wilting in acropetal succession. The toxic substances in the filtrate were not destroyed by boiling, this showing that they were thermostable. Both the filtrates gave a very positive test for nitrites with sulphanilic acid-*a*-naphthylamine.

Experiment VI.—This experiment was done to test the effect of dilution on the toxic action of the filtrate. The fungus was grown in Richards' solution for one week, and the filtrate obtained was diluted with distilled water (Table VI).

TABLE VI

Effect of dilution on the toxicity of the fungus filtrate

Filtrate diluted with water	Effect on seedlings after hours							
	4	8	24	28	32	48	72	124
Undiluted	F	F	D	W				
1 in 1	F	F	Dc	D	D	D	W	
1 in 3	T	T	T	F	F	D	W	
1 in 5	T	T	T	T	F	F	D	W
1 in 8	T	T	T	T	T	T	T	T

'F' means flagging, 'D' drooping, 'Dc' drooping of cotyledons, 'W' wilted, and 'T' turgid.

Sann-hemp plants placed in the undiluted filtrate showed a slight flagging after four hours, but wilted within twenty-eight hours. When the filtrate was diluted with an equal quantity of distilled water, plants did not wilt until after seventy-two hours and the period of drooping was much prolonged. The further dilution of the filtrate merely delayed the appearance of ill effects in the plants, until the dilution of one in eight possessed no toxic action. Control plants in Richards' solution and in the solution diluted with an equal quantity of water first became languid, but later regained turgidity ; in higher dilutions of Richards' solution plants did not show the initial flagging. After 124 hours control plants were still healthy, but were showing evidence of loss of vigour.

The above experiments indicate that, when *Fusarium vasinfectum* is grown in a culture solution containing inorganic nitrate, the fungus produces substances that are toxic to sann-hemp seedlings when grown in the filtrate and also inhibit seed germination. These experiments further bear out the evidence brought forth by Rosen [1926] in the case of the cotton wilt fungus that an inorganic salt in the form of nitrite is one of the toxic ingredients produced in the liquid substrate in which the fungus had grown, although Kulkarni and Mundkur [1928] were unable to determine appreciable quantities of nitrite nitrogen in the culture filtrate sufficient to induce wilting. This property of reducing inorganic nitrate to nitrite has also been shown to be possessed by the spores of the sann-hemp wilt fungus. Further, filtrates have been found to possess toxic properties : these are not destroyed by boiling but their toxicity was reduced by the dilution of the filtrate.

CROSS-INOCULATION EXPERIMENTS

Fusarium vasinfectum is generally considered to be restricted in its parasitism and there is also no definite evidence of selective parasitism in the different strains of this fungus. In Uganda Small [1920, 1922 and 1925] found *Fusarium udum*, now recognised as a strain of *F. vasinfectum*, attacking not only pigeon-pea, *Cajanus indicus*, but a variety of other plants. Briant and Martyn [1929] in Trinidad, however, were unable to secure inoculation of pigeon-pea with the sann-hemp wilt *Fusarium*, and Thorold [1931] further found cotton to be immune from the latter fungus. Recently Mitra [1934] has brought forth evidence that under the conditions at Pusa wilts in sann-hemp and in pigeon-pea are caused by similar physiologic strains of *F. vasinfectum*. Although the isolates from these two hosts were able to cross-inoculate, they always failed to infect cotton and *vice versa*. In view of this conflicting evidence, a series of experiments was planned to test the validity of the conception that *Fusarium vasinfectum* shows a high degree of specialisation in parasitism.

Stems of wilted sann-hemp and pigeon-pea plants grown in Pusa and its neighbourhood were secured through the kindness of Dr. M. Mitra, and from these single spore isolations of *Fusarium vasinfectum* were made. Similar cultures were made from the local wilted material obtained from Manjri and Arbhavi, and an isolate of the fungus from cotton was secured from Dharwar. All cultures were multiplied in Richards' solution, and the inoculum was mixed separately with steam-sterilised soil. The seeds of a wilt-resistant type, T 80, and of a susceptible type, T 5, of pigeon-pea were secured from the Imperial Economic Botanist for the experiments, since Mitra [1934] had used seeds of these types in his cross-inoculation studies. In all cases seeds were surface-sterilised before sowing in galvanised iron cans containing inoculated soil. Sterile water was used to water plants, and all possible precautions were taken to avoid contamination. Unless otherwise stated, these tests were made in soil temperature tanks of Wisconsin type held at 26° to 28°C., the optimum range for the development of wilt in sann-hemp.

Experiment I.—This experiment was done to determine whether the sann-hemp wilt *Fusarium* passes to pigeon-pea and *vice versa*. The results are recorded in Table VII.

TABLE VII

Results of cross inoculation of sann-hemp and pigeon-pea with strains of Fusarium vasinfectum *isolated from these hosts*

Serial No. of test	Source of inoculum	Pigeon-pea			Sann-hemp
		Type 80	Type 5	Local	
1	Manjri sann *Fusarim*	..	..	*8/0	15/15
2	Ditto	..	..	4/0	17/17
3	Ditto	15/0	11/0	14/0	5/5
4	Ditto	14/0	13/0	13/0	12/11
5	Ditto	15/0	11/0	12/0	22/20
6	Ditto	27/0	..	..	..
7	Ditto	..	17/0	.	..
8	Ditto	..	..	7/0	..
9	Pusa sann *Fusarium*	..	14/0	..	18/16
10	Ditto	..	17/0	.	15/15
11	Ditto	..	17/0	..	16/15
12	Stems of wilted sann plants (Manjri).	19/0	9/0	11/0	17/15
13	Ditto	..	14/0	..	43/42
14	Arbhavi pigeon-pea *Fusarium*	43/43	..	..	..
15	Ditto	..	31/29	..	..
16	Ditto	..	..	12/8	..
17	Ditto	..	..	18/18	21/0
18	Ditto	11/8	11/5	12/7	9/0
19	Pusa pigeon-pea *Fusarium*	12/6	12/6	11/7	11/0
20	Ditto	13/5	12/11	13/6	17/0
21	Ditto	5/2	3/2	10/4	4/0
22	Ditto	5/1	2/2	10/4	6/0

* Numerator denotes the number of plants exposed to infection, denominator the number of plants that wilted.

1-8.—In these tests a monoconidial culture of the fungus isolated from wilted sann-hemp plants collected at Manjri was used for inoculation of soil. In the first five tests (1-5) seeds of sann-hemp and pigeon-pea were sown together in containers of inoculated soil, and in the other three (6-8) only pigeon-pea was grown. In all seventy-one plants of T 80, fifty-two of T 5 and fifty-eight of local pigeon-pea were grown in inoculated soil for a period of seventy days ; but all remained healthy and isolations from these plants were sterile. However, of seventy-one sann-hemp plants exposed to infection, sixty-eight died of *Fusarium* wilt within forty days.

9-11.—These tests were similar to the previous ones except that the fungus (a single-spore culture) used for soil inoculation was isolated from wilted sann-hemp plants obtained from Pusa. Sann-hemp and pigeon-pea were grown separately in containers of inoculated soil, and there were in all forty-nine plants of sann-hemp and forty-eight of pigeon-pea, T 5.

Wilt appeared in sann-hemp ten days after sowing, and most of the plants were dead within forty days. Pigeon-pea plants were, however, allowed to stand over for another month, and were then uprooted and examined for vascular discoloration. No case of infection of pigeon-pea was noticed, and isolations made from these plants also remained sterile.

12-13.—In his cross-inoculation experiments, Mitra [1934] had used 'sann-hemp wilted material of the previous year' as source of inoculum. A similar experiment was done, in which stems of wilted sann-hemp plants were used to inoculate steam-sterilised soil. Seeds of sann-hemp and pigeon-pea were sown together in containers, and at the end of forty days fifty-seven out of sixty sann-hemp plants had died of *Fusarium* wilt, whilst all the pigeon-pea plants were healthy even after seventy days.

This experiment shows that the strain of *Fusarium vasinfectum* causing wilt in pigeon-pea is not common in Manjri soil.

14-18.—A single-spore culture of the fungus isolated from wilted pigeon pea plants collected at Arbhavi was used as a source of inoculum in these tests In three tests (14-16) only pigeon-pea was grown, whilst in the other two (17-18) sann-hemp and pigeon-pea were grown together. Wilting commenced fifteen days after germination, and plants were under observation for over two months. Of the 138 plants of pigeon-pea, 118 succumbed to *Fusarium* wilt comprising fifty-one plants of T 80, thirty-four of T 5 and thirty-three of local pigeon-pea. All the thirty plants of sann-hemp grew normally and, when uprooted and examined, showed no discoloration ; isolations from these plants also remained sterile.

19-22.—These tests were similar to the previous ones (14-18) except that the fungus used as inoculum was isolated from stems of wilted pigeon-pea obtained from Pusa. Sann-hemp and pigeon-pea were grown together in inoculated soil. After a period of two months fifty-six out of 108 plants of pigeon-pea had died of *Fusarium* wilt, comprising fourteen plants of T 80 and twenty-one each of T 5 and local. None of the thirty-eight sann-hemp plants showed any sign of the disease.

The results of these experiments are contrary to those of Mitra [1934] and indicate that wilts in sann-hemp and in pigeon-pea are not caused by similar physiologic strains of *Fusarium vasinfectum*, since in cross-inoculation tests the form on sann-hemp failed to pass to pigeon-pea and *vice versa*. In his cross-inoculation experiments, Mitra [1934] did not seem to have taken special care to avoid contamination as *Rhizoctonia* was often isolated as a 'secondary' parasite. Moreover, the use of wilted material either of sann-hemp or of pigeon-pea 'of the previous year' as a source of inoculum in cross-inoculation tests is open to serious objection, considering that the strains of the fungus causing wilt in these two hosts exist as saprophytes in Pusa soils. In his manurial experiments, McRae [1933] has shown that green manuring of sann-hemp shows the definite tendency to decrease the wilt in pigeon-pea at Pusa. If wilts in sann-hemp and in pigeon-pea were caused by similar physiologic strains of *Fuisarum vasinfectum,* one would expect green manuring to intensify rather than lessen wilt infection in a plot in which a crop of pigeon-pea had previously suffered from wilt.

Experiment II.—In 1933 three species of *Crotalaria,** *C. striata, C. anagyroides* and *C. usaramoensis,* were grown in the sann-hemp wilt-infected plot at Manjri. The course of wilt development in the three species was similar to that of sann-hemp, and by the end of the season all the plants were dead.

In 1934 tests were made in pots containing soil artificially inoculated with the sann-hemp wilt fungus. The seeds of *Crotalaria* spp. were disinfected in a solution of 1 in 1,000 mercuric bichloride before sowing. Germination was very poor in *C. usaramoensis.* Wilt appeared in *C. anagyroides* nine days after sowing, whilst it was not noticed until one month in *C. striata* and four months in *C. usaramoensis.* The results are recorded in Table VIII.

TABLE VIII

Cross inoculation of species of Crotalaria *with the sann-hemp wilt fungus*

Species of *Crotalaria*	No. of plants exposed to infection	No. of plants wilted
Crotalaria anagyroides	48	48
Crotalaria striata	34	33
Crotalaria usaramoensis	8	4

In another test disinfected seeds of *Crotalaria juncea* and *C. anagyroides* were sown in soil inoculated with the wilt fungus isolated from the latter host and held in soil-temperature tanks at 26° to 28° C. About one and a half months after sowing, seventeen out of nineteen plants of *Crotalaria juncea* and seven out of eight plants of *C. anagyroides* had died of wilt. On the 15th January 1935, seeds of T 80, T 5 and local pigeon-pea were sown in the same soil together with another batch of seed of *C. anagyroides* and *C. juncea.* All plants of *Crotalaria* spp. wilted, whilst pigeon-pea remained perfectly healthy until the 6th April when the experiment was concluded.

These experiments show that *Fusarium vasinfectum* from sann-hemp readily infects *C. striata, C. anagyroides* and *C. usaramoensis ;* but, like the sann-hemp strain, the isolate from *C. anagyroides* failed to pass to pigeon-pea.

---

* The seeds of *Crotalaria anagyroides* and *C. usaramoensis* were kindly supplied by Dr. N. B. Mendiola, Head of the Department of Agronomy, College of Agriculture, Philippines, and of *C. striata* by the Range Forest Officer, Kodaikanal, Madras Presidency.

Experiment III.—In the uniformly sann-hemp wilt-infected plot at Manjri, cotton was grown in 1933. All cotton plants remained healthy till the end of the season. Again in 1934, cotton was sown in pots containing soil inoculated with the sann-hemp wilt fungus ; but none of the plants showed any symptoms of wilt. Similarly, sann-hemp remained free from infection in soil inoculated with *Fusarium vasinfectum* from cotton. In both cases, however, control plants died of wilt.

These results are in accord with those of Thorold [1931] and of Mitra [1934] and indicate that the physiologic strain of *Fusarium vasinfectum* on cotton does not pass to sann-hemp and *vice versa.*

SEED TRANSMISSION OF WILT

It seems to be generally assumed that many *Fusaria* causing wilt diseases of plants are disseminated by seed. Orton [1931] in 1931 published a bibliography of seed-borne parasites, and since then many cases of seed dissemination of vascular *Fusaria* have been investigated. Kendrick [1931, 1934] has shown seed transmission of *Fusarium* wilt of cowpea and yellows of beans, and Snyder [1932] and Kadow and Jones [1932] have obtained similar evidence for wilt in peas. Taubenhaus and Ezekiel [1932] have experimentally proved that cotton wilt is transmitted by dead seeds infected by *Fusarium vasinfectum* as well as inside the viable seeds. Later, Kulkarni [1934] also proved the seed-borne character of the cotton wilt fungus. It seemed desirable, therefore, to discover whether the sann-hemp wilt fungus was carried internally in the seed or on its surface.

Experiment I.—Pods were picked from eighty-seven sann-hemp plants infected with *Fusarium* wilt in the infected plot at Manjri, and were divided into mature and immature lots. The seed was harvested by carefully opening the pods by hand so as to avoid contamination, and was examined for the presence of the fungus on the seed-coat. Seeds bearing pink fungus efflorescence were considered to be diseased. It will be seen from the following statement that immature pods contained a larger number of diseased seeds than the mature :

Pods	Total seeds	Diseased seeds
279 (mature)	2,310	131
151 (immature)	1,167	455

Experiment II.—In 1930 bulk seed was harvested by picking pods from a heavily diseased plant and opening them by hand so as not to contaminate the seed. Thirteen seeds were selected from this lot for further examination, and of these six were surface-sterilised with one in 1,000 mercuric bichloride solution for ten minutes. Each of these seeds was then placed with a healthy, disinfected seed on cotton wad in Roux tubes containing Knop solution. The following statement gives the results obtained :—

Seeds sterilised

1. Non-viable ; no fungus growth.
2. Ditto.
3. Ditto.
4. Germinated ; no fungus growth.
5. Germinated ; seedling wilted.
6. Non-viable; fungus grew out from dead seed and wilted the other seedling from healthy seed.

Seeds not sterilised

7. Non-viable ; fungus grew out from dead seed and wilted the other seedling from healthy seed.
8. Ditto.
9. Ditto.
10. Ditto.
11. Germinated ; seedling wilted.
12. Ditto.
13. Ditto.

These results bring out two points : (1) the sann-hemp wilt fungus may be carried internally in the seed or externally on the seed-coat, and (2) the non-viable seed harbours infection and may be an important source of transmission.

Another test was carried out in which eighteen seeds were selected from a quantity of seed harvested from diseased plants known to be infected by wilt, and of these eight were surface-sterilised. These sterilised seeds were planted on sterile cotton wool in Roux tubes containing Knop solution ; only one seedling wilted, from which *Fusarium* was recovered and successfully re-inoculated on sann-hemp seedlings grown in steam-sterilised soil. Typical wilt appeared in seedlings from untreated seeds in Roux tubes, showing that the fungus was probably carried mainly on the seed-coat.

Experiment III.—The work was continued in 1933 with 284 diseased plants selected from the thoroughly infected plot at Manjri. Bulk seed was harvested by opening pods by hand, and in all 8,786 seeds were obtained. The seeds were washed in running water, disinfected in one in 1,000 mercuric bichloride solution and finally rinsed in several changes of sterile water. These seeds were then germinated in Petri dishes lined with sterile, wet cotton wool. After one week the seedlings were cut open longitudinally under aseptic conditions and examined for discoloration of the vascular tissue. One hundred and eighteen seedlings showed internal browning, and *Fusarium* was recovered from discoloured portions and proved to be pathogenic on sann-hemp plants grown in sterilised soil. This evidence clearly indicates that the fungus may be carried in a very small percentage of cases inside the seed as dormant mycelium.

Experiment IV.—It is generally observed that many pods borne on heavily diseased plants are imperfectly developed and that such pods usually show pink efflorescence on their surface. An experiment was done to trace the course of the fungus in diseased plants and to ascertain whether pink efflorescence on pods was an indication of the fungus having entered the pods through flower stalks and peduncles. Two badly diseased plants, XIX-12 and XIX-4, were selected for this purpose. Short pieces of stem, flower stalk and peduncle were cut aseptically in each case and incubated to recover *Fusarium* in isolation.

From plant XIX-12, the fungus was recovered from the main stem and from a length of about thirty inches up the central flower stalk. In the other plant, XIX-4, the fungus could not be traced up the stem beyond two to three inches above ground level, although this plant appeared as badly diseased as plant XIX 12. The following statement gives the results of the examination of peduncles and flower stalks for internal infection :

Plant	Part examined	Sann-hemp *Fusarium* recovered	Saprophytic *Fusaria* recovered
XIX-12	70 peduncles	4	36
XIX-4	77 peduncles	0	0
XIX-12	62 side flower stalks . . .	5	0
XIX-4	70 side flower stalks . . .	0	0

It will be seen from these results that plant XIX-4, though diseased, did not harbour the fungus in its aerial parts, whilst the fungus was easily recovered from plant XIX-12, in which case it had ascended a considerable distance up the central flower stalk whence it had travelled through side branches to peduncles and pods. It is therefore obvious that seeds from a diseased plant may not necessarily harbour infection. However, when the fungus has finally reached the pods, it may grow out on their surface and on the seed under favourable environmental conditions, appearing as pink efflorescence.

This experiment has been repeated with similar results.

Summarising from the above experiments, it may be concluded that *Fusarium* wilt of sann-hemp is seed-borne. Sann-hemp wilt is normally transmitted externally on the seed, a very small percentage of which may also harbour internal infection as dormant mycelium. Infection of pods from peduncles often results in their development being retarded, and seeds formed in such pods are usually shrivelled and non-viable. These dead seeds often carry infection internally and are also an important source of wilt transmission.

SUMMARY AND CONCLUSIONS

The study of the wilt in sann-hemp caused by *Fusarium vasinfectum* Atk. shows it to be a widespread disease on the Deccan Canals, resulting in heavy reduction in the yield of green dressing per acre. Wilt is a serious menace to the Manjri Standard Method of sugarcane cultivation, in which sann-hemp, cane and cotton form a normal rotation.

Under field conditions yellowing of leaves is the first symptom and starts with the oldest leaves and gradually proceeds upwards. Concurrent with the yellowing of leaves, the petiole reveals a brownish discoloration of the vascular tissue. If infection occurs early, young seedlings may die in a few hours after emergence from the soil. When older plants are affected, wilting occurs slowly and these plants collapse in two to three weeks. Wilting is very rapid in September and October, but plants wilt gradually early in the season in June and July.

The morphological and cultural characters of the fungus, which is considered to be a strain of *Fusarium vasinfectum* Atk., have been described. There is no evidence of selective parasitism in the fungus since isolations secured from widely separated localities in the Bombay Presidency proved almost equally pathogenic on sann-hemp.

The sann-hemp wilt fungus makes the best vegetative growth in culture at 25° to 30° C., and wilt also makes the maximum development in a similar range of soil temperature, thus indicating a close parallelism between the development of the disease and the vegetative growth of the parasite. Wilt is also very destructive to sann-hemp in soil of low moisture content but, since soil temperature does not vary widely under the normal range of field conditions during the monsoon, soil temperature generally operates as the limiting factor in determining the severity of sann-hemp wilt.

Fusarium vasinfectum on sann-hemp produces the enzymes, emulsin, lipase, erepsin, trypsin and amidase, but there was no evidence that the fungus could utilise cellulose or starch.

It has been shown that the growth of the fungus in culture media containing inorganic nitrogen effects the reduction of nitrates to nitrites, but no such action was noticeable in media with organic nitrogen such as sodium asparaginate. The filtrates of twenty-four to twenty-eight days old cultures on Richards' solution were found to contain nitrites in quantities ranging from 0·0015 to 0·0022 mg. of nitrogen per c.c. of solution. Further, they were found to possess toxic properties : these were not destroyed by boiling but their toxicity was reduced by the dilution of the filtrate.

Fusarium vasinfectum on sann-hemp readily passed to *Crotalaria anagyroides*, *C. striata* and *C. usaramoensis* in cross-inoculation tests, but invariably failed to infect *Cajanus indicus* and cotton. There is thus no ground for Mitra's [1934] suggestion that wilts in sann-hemp and in pigeon-pea are caused by similar physiological strains of *Fusarium vasinfectum.*

Experimental evidence has been produced that *Fusarium* wilt in sann-hemp is seed-borne. Wilt is normally transmitted externally on the seed, a very small percentage of which may also harbour internal infection as dormant mycelium. Infected seeds are often shrivelled and non-viable, and such seeds may carry infection internally and are an important source of wilt transmission.

REFERENCES

Brient, A. K. and Martyn, E. B. (1929). *Trop. Agric.* (*Trinidad*) **6**, 258-260.

Chibber, H. M. (1914). *Bombay Dept. Agric. Bull.* **65**.

Clayton, E. E. (1923, 1). *Amer. Jour. Bot.* **10**, 71-88.

———— (1923, 2). *Amer. Jour. Bot.* **10**, 133-147.

Crabill, C. H. and Reed, H. S. (1915). *Biochem. Bull.* **4**, 30-44.

Gilman, J. C. (1916). *Ann. Miss. Bot. Gard.* **3**, 25-84.

Hansford, C. G. (1932). *Ann. Rept. Dept. Agric. Uganda, for the year ended* 31*st December* 1931 (*Part II*), 59-60.

Jones, L. R., Johnson, James and Dickson, J. G. (1926). *Wis. Agric. Expt. Stn. Res. Bull.* **71**.

Kadow, K. J. and Jones, L. K. (1932). *State College Washington Agric. Expt. Stn. Bull.* **272**, 1-30.

Kendrick, J. B. (1931). *Phytopath.* **21**, 979-983.

———— (1934). Abst. in *Phytopath.* **24**, 1139.

Kulkarni, G. S. (1934). *Ind. Jour. Agric. Sci.* **4**, 976-1048.

Kulkarni, G. S. and Mundkur, B. B. (1928). *Mem. Dept. Agric. India* (*Bot. Ser.*) **17**, 26.

Martyn, E. B. (1933). *Kew Bull. Misc. Inform.* 107-110.

McRae, W. and Shaw, F. J. F. (1933). *Imp. Counc. Agric. Res. Sci. Monograph* **7**

Mitra, M. (1934). *Ind. Jour. Agric. Sci.* **4**, 701-714.

Mitra, M. and Kheswalla, K. F. (1935). *Proc. Indian Acad. Sci.* **2**, 495-499.

Mundkur, B. B. (1935). *Ind. Jour. Agric. Sci.* **5**, 609-618.

Orton, C. R. (1931). *West Virginia Agric. Expt. Stn. Bull.* **245**, 1-47.

Reinking, O. A. and Wollenweber, H. W. (1927). *Philip. Jour. Sci.* **32**, 103-253.

Ridgway, Robert (1912). "Colour standards and colour nomenclature." 53 coloured plates, 1115 named colours.

Rosen, H. R. (1926). *Jour. Agric. Res.* **33**, 1143-1162.

Ryker, T. C. (1935). *Phytopath.* **25**, 578-600.

Small, W. (1920). *Kew Bull. Misc. Inform.* 321-328.

———— (1922). *Kew Bull. Misc. Inform.* 269-291.

———— (1925). *Kew Bull. Misc. Inform.* 118-126.

Snyder, W. C. (1932). *Phytopath.* **22,** 253-257.

Taubenhaus, J. J. and Ezekiel, W. N. (1932). *Sci. N. S.* **76,** 61-62.

Thorold, C. A. (1931). *Trop. Agric.* (*Trinidad*) **8,** 176-177.

Tims, E. C. (1926). *Jour. Agric. Res.* **33,** 971-992.

Tisdale, W. B. (1917). *Phytopath.* **7,** 356-360.

——————— (1923). *Jour. Agric. Res.* **24,** 55-86.

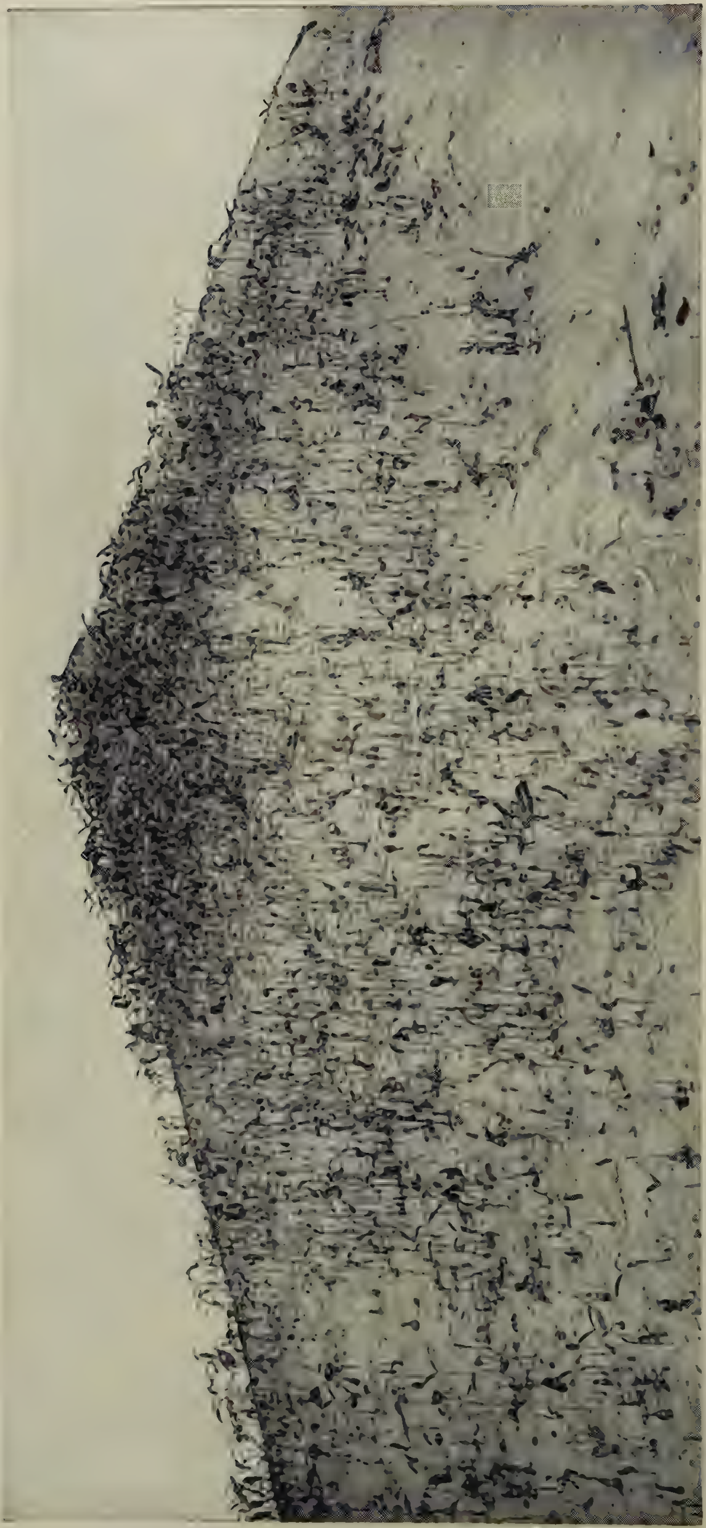
Van Hall, C. J. J. (1925). *Meded. Inst. voor Plantenziekten* **67** (ex. *Rev. Appl. Mycol.* **4,** 594, 1925).

Vincens, F. (1921). *Bull. Agric. de l'Instit. Scient. de Saigon* **3,** 381-384 (ex. *Rev. Appl. Mycol.* **1,** 294, 1922).

Wollenweber, H. W., Sherbakoff, C. D., Reinking, O. A., Johann, Helen and Bailey, Alice A. (1925). *Jour. Agric. Res.* **30,** 833-843.

Wright, J. and Leach, R. (1931). *Trop. Agric.* (*Trinidad*) **8,** 151-160.

PLATE XXXIV



Sann-hemp seedlings infected with *Colletotrichum curvatum* under field conditions.

AN ANTHRACNOSE DISEASE OF SANN-HEMP

BY

M. MITRA, Ph.D. (Lond.), D.Sc. (Lond.), D.I.C.
Imperial Agricultural Research Institute, New Delhi

(Received for publication on 8th March 1937)

(With plates XXXIV-XXXVII)

CONTENTS

		Page
I.	Introduction	443
II.	Symptoms	444
III.	Morphology	444
IV.	Isolation and parasitism	445
V.	Growth in culture	446
VI.	Historical and taxonomy	446
VII.	Methods of control	448
VIII.	Conclusion	449
	References	419

I. Introduction

A seedling disease of sann-hemp causing considerable damage was noticed for the first time at Pusa during August 1935, and again in 1936. Varieties sown before the rains escaped the disease but the same varieties sown in adjacent plots during August, when the weather was cloudy and accompanied by constant rain, were heavily affected by the disease, especially in fields where sann-hemp was thickly sown. The infection was so severe that practically all the seedlings died (Plate XXXIV). The disease gradually spread to the neighbouring plots where the plants were older, but the damage done to these was comparatively slight. Younger plants are more susceptible to the disease than are older plants. The microscopic examination of infected seedlings revealed the constant presence of a species of *Colletotrichum*.

The spattering of rain helps in the distribution of the disease. At the time when humidity is high the fungus produces multitudes of spores which if undisturbed, tend to cling together even when dry. The spore masses are rapidly broken by falling rain and are washed to the healthy parts of the plant or to the ground whence they may be carried to other plants by spattering water. A light shower falling during a period of active spore production appears to be responsible for a large part of the spread of the disease from isolated centres.

II. SYMPTOMS

The seedlings are attacked first on the cotyledons, and infection spreads later to the stem and the growing point. The disease first makes its appearance in the form of soft discoloured areas on the cotyledons. Later brownish spots are formed on all parts of the host except the underground roots. Infected seedlings droop from a point below the cotyledons. The affected cotyledons themselves droop from the petiole. The infection spreads downwards and within forty-eight hours acervuli are formed with copious spore formation on the infected area. The young seedling when infected generally dies as the growing point is attacked. When a rather older plant is infected the disease is restricted to spots on leaf and stem and the badly infected leaves fall off. If the weather is dry and bright the plant escapes the disease, the infected leaves fall down and the newly formed leaves remain healthy.

The spots on older plants first appear on one side of the leaf but gradually enlarge and extend to the opposite side. These spots are greyish brown to dark brown, roundish or irregular. Several spots join and infect a large area They are also formed on the mid-rib. As the seedlings grow they gradually become resistant to the disease. The prevalence or severity of the disease is influenced by temperature, moisture in soil and air, rain and dew. Vacant patches occur in the plot where the seedlings have died or completely disappeared. Plate XXXIVgives a general idea of the virulence of the disease and Plate XXXV shows healthy and diseased seedlings for comparison.

III. MORPHOLOGY

The mycelium is at first localized in the tissue of a lesion either on leaf or stem of a young seedling, and in mild cases does not extend internally to other parts. In severe cases it not only penetrates the cortical region but also invades the vascular bundles and the infected parts soon decompose, weakening the plant, which later droops from the point of attack.

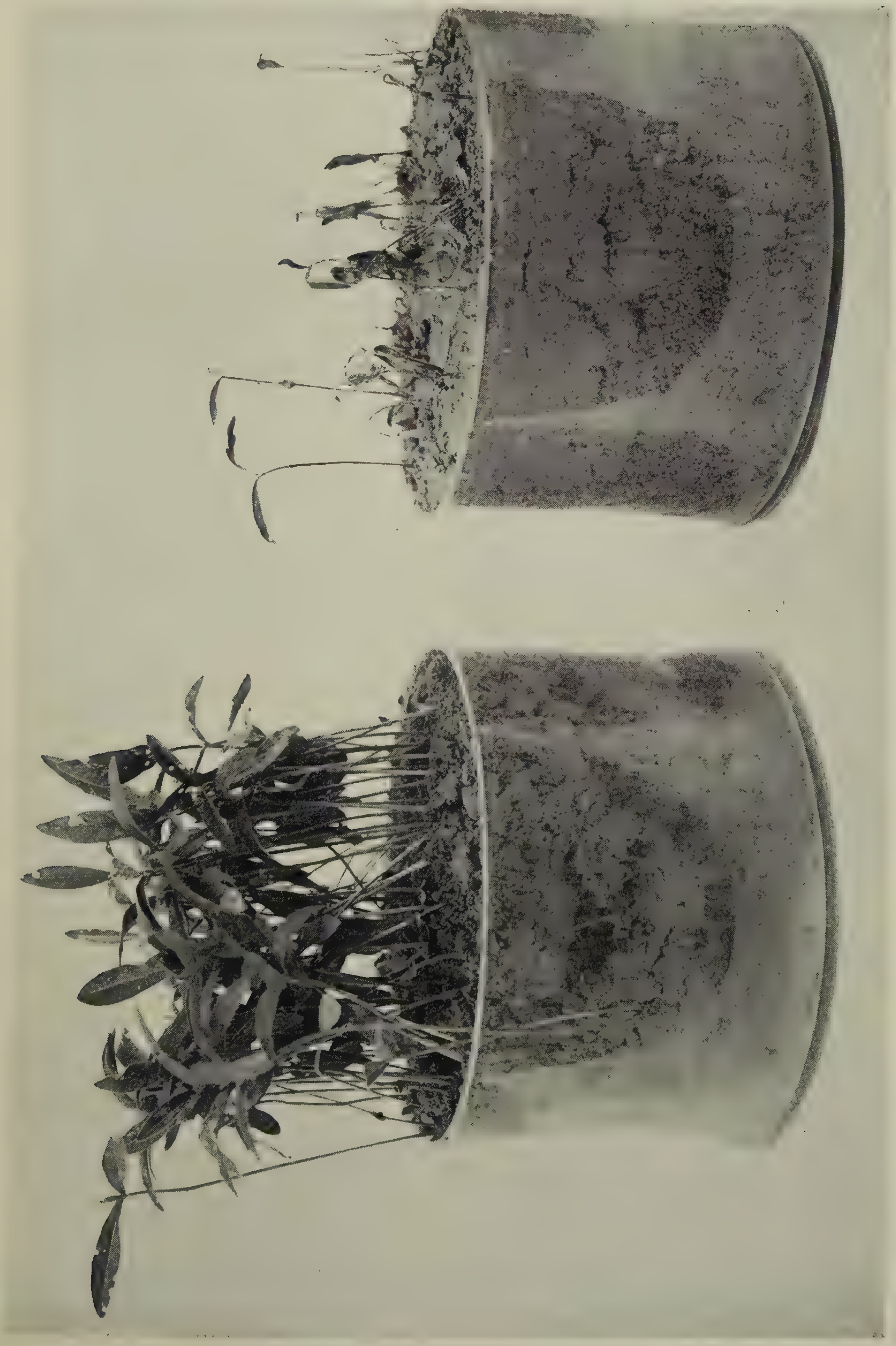
The acervuli are formed in abundance in the epidermis of the diseased area. They are white or faint pinkish in colour and consist of simple, erect, closely packed conidiophores, and setæ.

From the tip of the conidiophores the conidia are budded off apically, one at a time, and pile up in a light pinkish heap on the top of the acervulus. The conidia are held together by a mucilaginous secretion.

The conidia are one celled, hyaline, falcate, and acute at each end. In size they are 15-24$\mu \times$ 3-4 μ, the average being 18·64 $\mu \times$ 3·03 μ. The conidia on germination produce a germ tube which forms an appressorium.

The setæ are brown to dark brown in colour, septate, tapering towards the tip and swollen at the base. They are formed between the conidiophores and in size are 66-140 $\mu \times$ 4-6 μ with an average of 104·64 $\mu \times$ 4·98 μ. Plate XXXVI shows conidia and acervuli.

PLATE XXXV



Diseased sann-hemp seedlings compared with healthy seedlings.

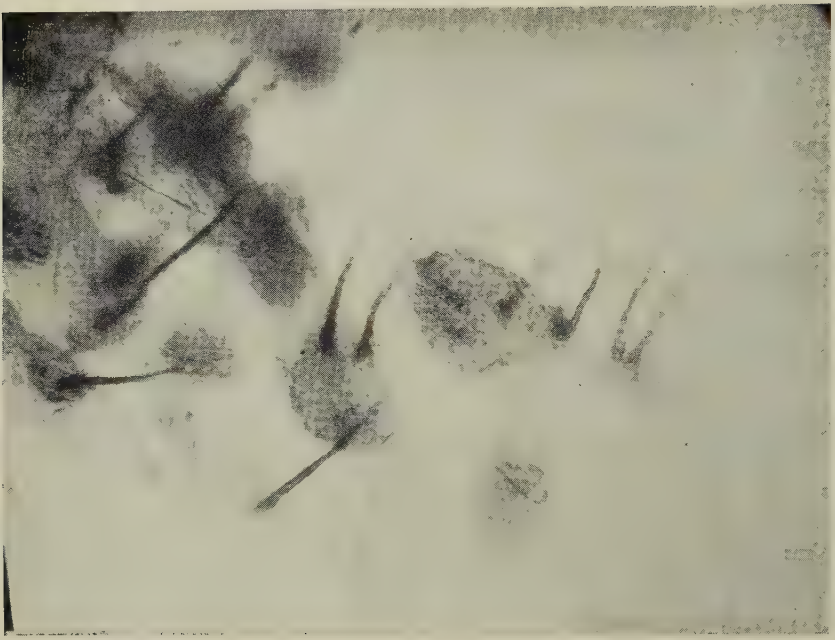


Fig. 1. Acervuli and setae of *Colletotrichum curvatum* (×500)

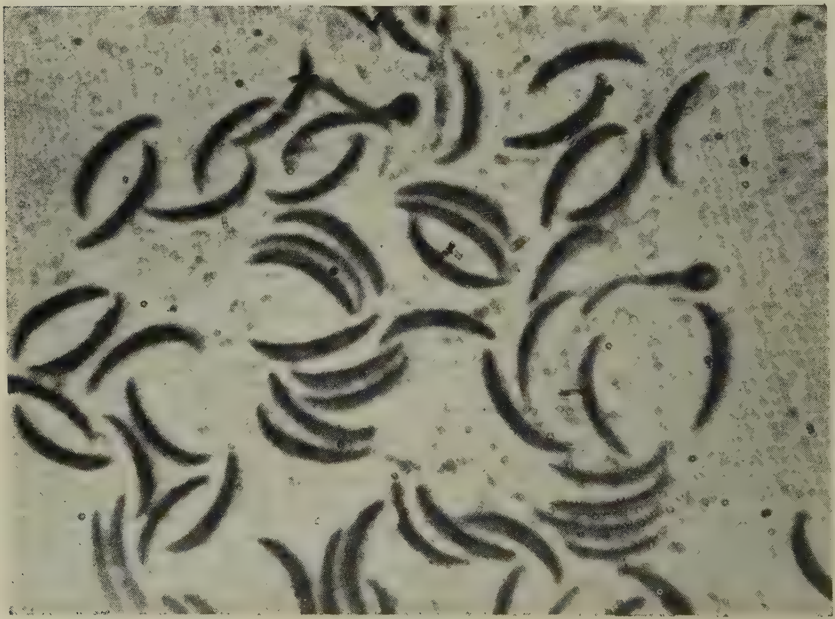


Fig. 2. Conidia of *Colletotrichum curvatum* (×1,000).

IV. Isolation and parasitism

Several isolations made from cotyledon, leaf and stem showed the presence of a species of *Colletotrichum*. The fungus was brought into pure culture and several inoculations were made on sann-hemp. Re-isolations proved identical with the parent culture.

The following infection experiments were carried out to prove the pathogenicity of *Colletotrichum* sp.

Experiment I.—Sixty-one young seedlings, 4″ in height, were inoculated by spraying with a spore suspension in water. Forty-three plants thus sprayed were kept in a moist chamber and the remaining eighteen were kept in shade. The first symptoms were noticed after forty-eight hours when all the pots with the seedlings were removed from the moist chamber and kept in the open. On the third day fructification of the fungus was noticed on all parts of the seedlings. Most of the plants died on the fifth day. The plants kept in the open showed a more severe attack, due to cloudy weather and rain. The spots were at first minute and gradually enlarged and became brownish to greyish brown in colour. Heavily infected leaves wilted rapidly. Later when the weather became dry and there was no rain the surviving plants gradually grew up into healthy plants showing however distinct marks of infection on lower leaves and stem.

Experiment II.—Twelve seedlings were inoculated by placing spores and mycelium on the stem and were kept in a moist chamber. After forty-eight hours the seedlings were drooping from the point of infection and died in four days.

Experiment III.—Spores with mycelium were placed on ten leaves and also on tender parts of the stem of a grown-up plant in ten places. The inoculated parts were kept moist by covering them with wet cotton wool. Infection took place only on two leaves, the stem being unaffected.

Experiment IV.—Twenty-two seedlings, 2″ high in pots, were inoculated by spraying with a spore suspension in water and were covered with bell jars. The first sign of infection was noticed after eighteen hours when six seedlings were found to be already dead. The growing point was infected and in some cases infection spread to the hypocotyl which showed signs of rotting. Within two to three days all the plants died.

Experiment V.—Fifty seedlings, 6″ in height in pots, were inoculated by spraying with a spore suspension. In three or four days all plants died.

Experiment VI.—Twenty leaves of a grown-up sann-hemp plant were inoculated with spores and mycelium and the inoculated spots were kept moist by placing wet cotton wool over them. Local discoloration of the tissue was produced but infection did not spread.

The infection experiments carried out proved that the fungus is a virulent parasite and that seedlings are susceptible while grown-up plants tend to resist the attack of the disease. Plate XXXVII shows healthy plants and those sprayed with a spore-suspension.

Cross inoculations.—Cross inoculations were made several times on leaves of seedlings and older plants of pigeon-pea, but no infection took place.

V. Growth in culture

The fungus grows very well on a large number of media and forms good aerial growth of greyish colour. It forms acervuli in large numbers which are scattered all over the medium. Generally the acervuli remain immature for a long period and conidia are formed only in old cultures. However, if a small bit of mycelium with acervuli is incubated in a moist chamber, conidia are formed within a day or two.

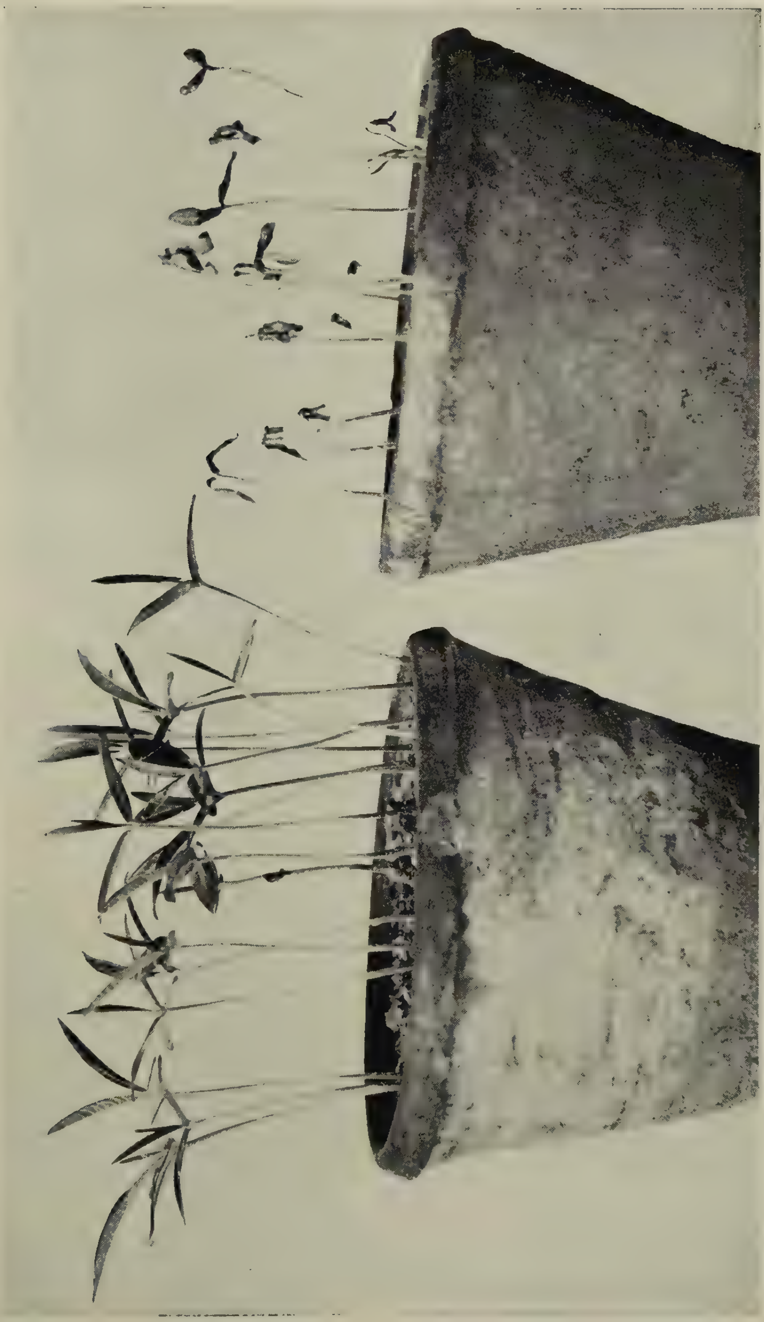
Chlamydospores are also formed in abundance especially on Dox's agar. These are formed by the thickening of mycelial cells which become brownish and are very much like appressoria. The fungus often formed a saltant which occurred in the form of a sector on plates of oat-meal agar. The saltant was isolated and compared with the parent culture. It differed from the parent culture in having dark greenish mycelium as compared to the light grey colour of the parent. It also formed zones when plates were exposed to alternate light and darkness. In other respects both the parent and saltant agreed.

Conidia when young are straight to slightly curved or bent on one side but with age become typically falcate like those formed in nature.

VI. History and taxonomy

Three anthracnose diseases of sann-hemp have been recorded. Petch [1916] described a new species of *Colletotrichum* from Peradeniya, Ceylon on *Crotalaria striata* and named it *C. crotalariæ* Petch. This fungus has also been recorded from the South of the U. S. A. by McKee and Enlow [1931] on *C. striata* and *C. spectabilis*. This anthracnose disease, according to these authors, does a good deal of damage and attacks the stem from soil level upwards; the bark is killed and sloughed off. The attacked plant gradually dies, shedding its leaves from the base upwards and turning brownish black.

Massee [1913] described *Glœosporium crotalariæ* as parasitic on young shoots of *C. juncea*. According to this author, circular brownish patches first appear on the young shoots, these gradually increase in size and encroach on one another, forming irregular patches. Just before the spore masses burst through the surface, the central portion of the patches presents a greyish appearance, due to the upraising of the cuticle. Brown patches appeared five days after the application of spores to the unbroken surface of the shoot. Briant and Martyn [1929] described another species of *Colletotrichum* and named it *C. curvatum*. According to these authors it is essentially a seedling disease and the amount of damage it causes is quite considerable, especially when the seed is thickly sown. The symp-



Sann-hemp seedlings sprayed with spore suspension together with control.

toms of the disease as described are very much similar to those of the disease of sann-hemp in Pusa, while the symptoms of the disease produced by *C. crotalariæ* and by *G. crotalariæ* are quite different.

In shape conidia of *C. crotalariæ* are cylindrical, with round ends, those of *G. crotalariæ* oblong to elliptical with ends rounded, whereas conidia of *C. curvatum* are falcate with acute ends and are similar to those of the fungus isolated at Pusa.

The size of conidia and setæ of the four anthracnose fungi recorded from sann-hemp are given below in the table. The data for Pusa strain are based on a measurement of 200 spores and setæ.

TABLE I

Length and width of conidia and setæ of four anthracnose fungi on sann-hemp

Fungus	Conidia				Setæ			
	Length (μ)		Width (μ)		Length (μ)		Width (μ)	
	Range	Average	Range	Average	Range	Average	Range	Average
Glœosporium crotalariæ.	25—28	..	7—8	..	..	..	..	..
Colletotrichum crotalariæ.	12—16	..	5	..	..	70	..	4
Colletotrichum curvatum.	16—22	19·8	3·6	3·6	90—160	..	4—6	..
Colletotrichum sp. (Pusa).	15—24	18·64	3—4	3·03	66—140	104·64	4·4—6	4·98

It will be noticed that the conidia of *G. crotalariæ* are the longest while those of *C. crotalariæ* are the shortest. The conidia of *C. curvatum* however approach in length those of *Colletotrichum* sp. (Pusa). In width the conidia of *G. crotalariæ* and *C. crotalariæ* are much broader than those of *C. curvatum* and *Colletotrichum* sp. (Pusa). The latter two very closely agree.

Similarly in length and width of setæ both *C. curvatum* and *Colletotrichum* sp. closely agree. Thus the anthracnose fungus on sann-hemp at Pusa closely agrees with *C. curvatum* from Trinidad both in shape and size of the conidia and setæ. Both the fungi attack seedlings. Hence *Colletotrichum* sp. occurring at Pusa may be considered as a strain of *C. curvatum* Briant and Martyn.

VII. CONTROL MEASURES

The following experiments were carried out to find out a suitable method for controlling the disease :—

(*i*) Control—untreated—seed-sown.

(*ii*) Seed was disinfected with 0·25 per cent aqueous solution of uspulun for thirty minutes, dried and sown.

(*iii*) Seed was heavily infected by soaking in a heavy suspension of spores in water for thirty minutes and was then dried and sown. Microscopic examination of dried seed showed the presence of spores on the seed-coat.

(*iv*) Seed was treated as in (*iii*) and then disinfected as in (*ii*) and sown.

For each treatment there were five pots and each pot had about twenty-five seedlings. On the fifth day after sowing, seedlings in (*iii*) showed signs of infection and on the sixth day all the seedlings showed heavy infection. All the seedlings in (*iii*) died on the seventh day, due to the infection spreading to the growing point. When the seedlings broke through the ground the fungus spores on the broken seed-coat came in contact with the expanding cotyledons which thus became infected. No disease appeared in (*ii*) and (*iv*) and in (*i*) there was slight infection. This shows that the disease may be seed-borne and disinfection of seed before sowing may check the disease.

Spraying against *Colletotrichum* on sann-hemp was also tried as follows :—

(*a*) Ten pots each with fifteen seedlings were placed in a moist chamber and were sprayed with spore suspension in water. After twenty-four hours duplicate pots were sprayed with—

(*i*) Water

(*ii*) 0·5 per cent Bordeaux mixture

(*iii*) 1·0 per cent Burgundy mixture

(*iv*) Shirlan (0·25 of Shirlan W. S. *plus* 0·1 per cent Agral.)

(*v*) Malachite-green (one in 10,000 aqueous solution *plus* 0·1 per cent Agral).

There was a heavy death-rate in all. Thus spraying with fungicides was found to be unsuitable for plants which were already infected.

(*b*) The experiment was again repeated but in (*ii*) one per cent Bordeaux was used and in (*iv*) one per cent Shirlan A. G. and Agral were used. After four days all plants wilted except in (*ii*) and these also wilted within next two days. Thus one per cent Bordeaux mixture was the only fungicide that slightly retarded attack in already infected plants.

(*c*) The spraying experiments as stated above were again tried but in (*v*) 0·25 per cent Shirlan without Agral was used and the fungicides were applied to seedlings twenty-four hours before spraying with spore suspension. More than half the number of plants under control and Shirlan died within two days but the seedlings treated with Bordeaux mixture, Burgundy mixture and Malachite-green showed less sign of disease and took ten days to die. Thus if seedlings are sprayed with fungicides before the infection, the attack is retarded.

VIII. CONCLUSION

A disease of sann-hemp causing serious damage to young seedlings was noticed for the first time at Pusa during August 1935. The causal organism was found to be *Colletotrichum curvatum* Briant and Martyn, previously recorded from the West Indies. The infection experiments carried out proved its pathogenicity. The disease is very virulent during the seedling stage of the host plant and especially if seed is sown late when the weather is moist and cloudy. The early-sown crop generally escapes the disease and with age the plant becomes less susceptible to infection.

Under favourable conditions of temperature and moisture the complete cycle from infection to spore production may occur within a few days. Conidia are produced abundantly even on small lesions and in periods of high humidity they are produced in enormous quantities on newly developed lesions as well as on dead plants. The most serious aspect of the disease is the local distribution of spores by spattering rain as the conidia are thus carried to the healthy parts of the plant or to other plants.

The treatment of seed with disinfectants checks the disease. Spraying with fungicides has been found to be helpful if carried out in dry weather.

The experiments on control measures by spraying with fungicides were carried out by Mr. L. D. Galloway, the Imperial Mycologist, and my grateful thanks are due to him for permission to use his data.

REFERENCES

Briant, A. K. and Martyn, E. B. (1929). *Trop. Agric.* **6**, 258-260.

Massee, G. (1913). XXXIII *Addition to the World Fauna and Flora of the Royal Bot. Gardens, Kew.* **14**, No. **6** (*Bull. Misc. Information Royal Bot. Gardens, Kew*, **198**).

McKee, R. and Enlow, C. R. (1931). *U. S. Dept. Agric. Cir. Bull.* **137.**

Petch, T. (1917). *Ann. Royal Botanic Gardens* (*Peradeniya*) **6**, 239.

A GENETICAL STUDY OF ROOTS IN RELATION TO DISEASE-RESISTANCE IN COTTON

BY

V. G. PANSE

Institute of Plant Industry, Indore, Central India

AND

A. F. PATEL

Agricultural Experimental Station, Baroda

(Received for publication on 26th January 1937)

CONTENTS

	Page
I. Introduction	451
II. Method of study	451
III. Experimental results	452
IV. Discussion	456
V. Summary	456
VI. Acknowledgments	457
References	457

I. Introduction

The present paper describes the results of a study of roots of cotton types grown at the Agricultural Experimental Station, Baroda, in the course of investigations on the root-rot disease (*Macrophomina* sp.) of Gujarat cottons. The object of the study was to discover if any genetical relationship exists between root characters and incidence of the disease and whether advantage can be taken of such a relationship, if it exists, in evolving resistant strains.

Weaver [1926] in his classical study of roots of field crops suggested selecting plants whose roots are resistant to fungus attack as a very promising method of procedure, but there are hardly any references in the literature to work on these lines. From an examination of root systems of cotton varieties, Kulkarni [1934] came to the conclusion that there was no relationship between the type of root system and resistance to wilt. His method of exposing roots was, however, very laborious and he was able to study only a few plants.

II. Method of study

Since root development is greatly influenced by environmental conditions, it is clear that roots of a large number of plants must be examined if conclusions are to be statistically sound. For this purpose a rapid method of exposing roots is necessary. The method adopted in the present study is described below :—

The plants selected for examination were profusely watered. The following morning a skilled labourer dug out the roots gently with a pick-axe. Beginning at a distance of about two feet from the stem he dug to a depth of four or five inches at a time and exposed the laterals by removing the soil with his hands till he reached the tap-root. Successive depths were thus dug out until the tap-root became so thin that it could not be traced further. With the help of a man to remove the soil, the labourer could expose roots of about thirty-five to forty plants a day. In spite of care, damage to laterals could not be avoided ; but a special effort was made to keep the tap-root intact.

For examination of the root-system the tap-root length was divided into three regions. The first two regions were of fifteen centimeters each and the third included the remaining length. The following characters were studied:

1. The total length of the tap-root.

2-4. The diameter of the tap-root in each of the three regions.

5-7. The total number of laterals in each of the three regions.

In the first experiment, lengths of thick, medium and thin laterals in each region were also measured ; but on account of the subjective nature of the classification and the high variability of the measurements caused by damage during digging, the examination was given up in later trials.

III. EXPERIMENTAL RESULTS

In 1934-35, roots were studied in two experiments, each consisting of two 5×5 Latin squares, laid out to compare the root-rot resistance of selected strains, with Broach 9, a susceptible variety, as control. The details of the lay-out have been described elsewhere [Hutchinson and Panse, 1935]. Statistical examination of the mortality, due to root-rot, showed that the strains KS and Rozi suffered significantly the least damage. No. 8, DS and BS were considerably more susceptible to root-rot than the above, but were significantly less attacked than Broach 9 while the strains I.32/24 and I.65/12 were as badly affected as Broach 9.

For examination of roots, two pairs of plants, each consisting of a plant of the strain and a plant of Broach 9 from the neighbouring row, were selected at random from each plot and the character examined was recorded as an average difference per plot between the strain and the control, with appropriate sign, the value for the control being always subtracted from that for the strain. Data for the complete Latin squares were, however, not available, because roots in the control plots were not examined. The differences were, therefore, reduced by Yates' method [1936] for the analysis of incomplete Latin squares. Mean differences for different characters with their standard errors and significance and also the significant difference for comparison between strains are given in Table I. The mean values given in the table were obtained only from ten plots each and their standard errors are high. The rows and columns of the Latin squares did not eliminate any appreciable portion of the total variation. Examination of a greater number of pairs per plot might have reduced the standard errors.

TABLE I

Mean differences in root characters between selected strains and Broach 9 (susceptible control)

Root character	Experiment I, 1934-35						Experiment II, 1934-35						Broach 9 plot, 1935-36	
	No. 8	I. 32/24	I. 65/12	Rozi	Standard error	Significant difference between strains	DS	BS	Rozi	KS	Standard error	Significant difference between strains	Broach 9	Standard error
Tap-root length, cms.	**10·2**	—1·8	—4·3	**14·2**	3·8	11·3	—2·4	0·4	**17·0**	**6·2**	2·3	6·9	2·7	2·8
Diameter of tap-root in first region, cms.	**0·22**	0·01	—0·13	**0·21**	0·09	0·28	0·14	—0·14	**0·48**	0·13	0·09	0·27	**—0·15**	0·06
Diameter of tap-root in second region, cms.	**0·19**	0·00	0·01	**0·33**	0·06	0·17	0·06	—0·07	**0·61**	*0·15*	0·07	0·22	—0·02	0·03
Diameter of tap-root in third region, cms.	**0·14**	—0·06	*—0·08*	**0·21**	0·04	0·12	—0·05	—0·01	**0·35**	**0·18**	0·06	0·17	0·07	0·04
No. of laterals in first region.	0·4	—1·5	—1·2	—0·5	1·3	...	—1·3	—2·1	*—3·3*	—2·3	1·6	...	**—3·6**	0·6
No. of laterals in second region.	—0·1	0·6	1·6	**2·4**	1·0	...	*1·9*	—1·2	**6·5**	**2·3**	1·0	3·1	*1·1*	0·5
No. of laterals in third region.	1·1	—0·8	—1·3	**2·7**	0·9	2·6	—0·1	—0·1	**2·8**	0·8	0·5	1·6	0·1	0·3

NOTE.—Mean differences for which P is $< 0·05$ printed in heavy type, those with P between 0·05 and 0·1 printed in *Italics* and the rest in ordinary type.

It will be seen from the table that the most resistant strains KS and Rozi and also No. 8, which is more resistant than the control show a strongly significant increment in total length of the tap-root over the control. Rozi and No. 8 had significantly thicker tap-roots than the control in all the three regions. KS had a significantly greater diameter of the tap-root than the control only in the third region, while the increment is suggestive in the second. Rozi had a significantly larger number of laterals than the control in the second and the third regions. KS showed a similar superiority over the control in the second region while DS showed a suggestive increment in that region. It is interesting to note that all strains except No. 8 showed a lower number of laterals in the first region than the control and for Rozi the difference was almost significant in the second experiment. The susceptible strains, I.32/24 and I.65/12, did not differ from the control in any character.

Comparison among strains shows similar differences between the more resistant and less resistant strains. In experiment I in tap-root length Rozi and No. 8 showed a significantly greater difference from control than the other two strains. Differences in the diameter of the tap-root were similar. In the number of laterals in the upper two regions differences were not significant, but in the third region Rozi differed significantly from the strains I.32/24 and I.65/12. In experiment II, Rozi was significantly superior to the other strains in all characters except in the number of laterals in the first region in which there were no significant differences. KS showed similar superiority over one or both of the remaining strains except in the number of laterals in the third region.

It is clear from these comparisons that a long tap-root and its profuse branching in the lower regions are characters associated with resistance to root-rot.

In 1935-36, a special plot of Broach 9 was grown for root study. The object of this study was to discover whether there was any relationship between root characters and resistance to root-rot within a strain. The plot was one-half acre (272 ft. × 78 ft.) in size and consisted of twenty-six rows three feet apart. Three or four seeds were sown in each hole, taken one foot apart in the row. After full germination two seedlings per hole were retained in alternate rows and only one seedling per hole in the remaining rows. An attempt was made to study the differences between plants which had been exposed to root-rot and had resisted, and plants which had not been so exposed. In the rows with two plants per hole, healthy plants were selected which were growing in holes in which one plant had died. Each of these was paired with the nearest healthy plant with healthy neighbours in one of the neighbouring rows. It was proposed to study a large number of such pairs, because differences in the root characters of the two kinds of plants, if they exist, would necessarily be small and must be determined with accuracy. Damage due to root-rot was, however, heavy and seventy-six per cent of the plants in the plot were killed. Only sixty-nine pairs, therefore, could be examined. The results are given in Table I.

The tap-root of the survivors was longer than that of the unselected plants but the difference was not significant. In the number of laterals in the second region, the difference was in favour of the survivors and approached significance ; but was negligible in the third region. The survivors had a significantly lower number of laterals in the first region and this confirms the indications obtained in the earlier experiment. It is interesting to note that the diameter of the tap-root of the survivors was significantly smaller than that of unselected plants in the first region and suggestively greater in the third region. In the earlier trial a greater diameter of the tap-root in the first region was generally associated with a longer tap-root ; but these results show that this is not always so. Measurement of the diameter cannot therefore be substituted for the measurement of the length of the tap-root.

In spite of the rather low number of pairs examined, it is clear that differences in certain root characters between resistant and susceptible plants exist within a strain and are generally similar to those observed in resistant varieties. The present results supply the additional information that resistant plants have a definitely lower number of laterals in the first region.

Roots were examined in the selection experiment in the same year. The experiment consisted of two 5×5 Latin squares laid out on the same lines as in 1934-35 and included the strains KS, No. 8, BS, DS and Broach 9 (control), all of which except the control had undergone further selection for one year. There were two plants per hole in this trial and roots of all plants which survived in the hole where the other plant died were examined. The corresponding plants from control rows were, however, omitted from examination and instead of examining differences it was necessary to analyse actual mean values per plot. This procedure would be expected to increase the variability of plot values due to the absence of local control and would narrow down the differences between the resistant strains and the control as all comparisons including those with the control were among plants selected for resistance. No significant differences were observed in tap-root lengths or in the number of laterals in the first region. The errors of the mean values were low, being 3·7 and 8·4 per cent respectively. The number of laterals in the lower two regions was analysed together and KS, the most resistant strain, was found to have a significantly higher number of laterals than BS or Broach 9. The strain DS which suffered equally to BS from root-rot had, however, an almost significantly higher number of laterals than BS. The standard error per cent of the mean was again 5·5 only.

It may be concluded from these experiments that there are significant differences in certain root characters between varieties with different degrees of susceptibility and also between resistant and unselected plants within the same strain. A long tap-root, profuse branching below 15 cm. and few laterals in the first 15 cm. of the soil are associated with resistance to root-rot.

IV. Discussion

Exposing roots by washing away the soil with a spray of water is the method generally followed for root examination by workers in India [Kulkarni, 1934]. Though it probably gives a more accurate picture of the root-system than the method adopted in the present study, it is a very slow and laborious process and Kulkarni required up to six days to expose the roots of an adult cotton plant. When it is necessary to study a large number of plants in a comparatively short time, speed is more important than the accuracy of the individual observation. In the present study, the root examination carried out in the two selection experiments, was done without any loss of crop from the plants studied. The examination was begun after harvesting was complete (at the end of March) and was finished within five weeks. This allowed just sufficient time for the analysis of the data before mid-May, when the next crop was sown. Even this method, therefore, was only just sufficiently rapid to provide results in time to be of value for selection work.

The discovery of significant differences in root characters between resistant and unselected plants within a strain is of considerable practical importance to the plant breeder. Significant differences between varieties in themselves cannot give any guidance for single-plant selection. It is possible that the association between varieties, of differences in root habit with differences in resistance may be fortuitous, and not the result of genetic linkage or physiological association. The evidence that similar associations between root characters and resistance exist within a strain effectually disposes of the possibility that they may be fortuitous, and demonstrates their value to the plant breeder. In making selections from the 1935-36 strains for subsequent trial at Baroda, root characters were taken into consideration and in choosing among plants with similar commercial characters, those with longer tap-roots, greater branching in the lower regions and less branching in the upper region were given preference.

V. Summary

A rapid method of exposing and examining roots of cotton plants is described.

A long tap-root, a large number of laterals in the lower regions and a small number of laterals in the upper region are shown to be associated with greater resistance of certain varieties to root-rot. Similar differences are shown to exist between resistant and unselected plants within Broach 9. Such differences within a strain justify single-plant selection based on desirable root characters.

VI. ACKNOWLEDGMENTS

The investigation here reported forms part of the Indian Central Cotton Committee's Baroda Root-Rot Scheme. The experiments were designed at the Institute of Plant Industry, Indore, and carried out at the Cotton Research Station of the Department of Agriculture, Baroda.

We are indebted to Mr. J. B. Hutchinson of the Institute of Plant Industry for guidance and criticism during the conduct of the experiments and the writing up of the data.

We are also indebted to the Commissioner of Agriculture, Baroda State, and Dr. V. N. Likhite, Officer-in-Charge, Cotton Root-Rot Scheme, Baroda, for facilities for carrying out the experiments.

REFERENCES

Hutchinson, J. B. and Panse, V. G. (1935). *Ind. Jour. Agric. Sci.* **5**, 554.

Kulkarni, G. S. (1934). *Ind. Jour. Agric. Sci.* **4**, 976.

Weaver, J. E. (1926). 'Root Development of Field Crops.' McGraw Hill Book Company, New York, p. 90.

Yates, F. (1936). *Jour. Agric. Sci.* **26**, 301.

STUDIES ON THE STINKING SMUT OR BUNT OF WHEAT IN INDIA

BY

M. MITRA, Ph. D. (Lond.), D. Sc. (Lond.), D.I.C.
Assistant Mycologist, Imperial Agricultural Research Institute, New Delhi

(Received for publication on 2nd March 1937)

(With two text-figures)

CONTENTS

		Page
I.	Introduction	459
II.	Effect of bunt (*Tilletia indica*) on wheat	460
III.	Control measures by seed treatments	467
IV.	Soil infection as a factor in the transmission of bunt	473
V.	Effect of climatic conditions on bunt	476
VI.	Conclusion	477
VII.	Summary	477
	References	478

I. Introduction

In a previous paper [Mitra, 1935] the author has established that *T. indica* Mitra is the cause of the bunt of wheat in India, but the manner in which loss occurs to the wheat plant due to this disease has not yet been recorded.

Several authors [Harwood, 1892; Potter and Coons, 1918; Sampson and Davis, 1927; Rodenhiser, 1931] have reported on the retarding influence on the development of the wheat plant, significant reduction in length of culms and height of the wheat plant due to the various species of *Tilletia*, whereas Selby [1898] and McAlpine [1910] have found no difference. In order therefore to determine the effect of *T. indica*, the bunt which is common in some parts of the Punjab and North-West Frontier Province, on the ears of wheat plants, lengths and the numbers of spikelets of healthy and bunt-affected ears of several varieties of wheat were noted and compared.

In order to determine the effect of some of the fungicides on the elimination of infection by *Tilletia indica* experiments were carried out at the Botanical Sub-Station, Karnal, during 1933-34. Copper carbonate, ceresan, formalin, and uspulun (universal) were used and their results have already been recorded [Mitra.

1935]. Experiments so far conducted lead to the conclusion that the percentage of infection can be considerably reduced by treatment of wheat seeds but that none of the fungicides is capable of completely checking the disease. To confirm the results reported in 1935 [Mitra, 1935] and further to study the effect of hot water treatment and of solar energy and sun-heated water recommended by Luthra and Sattar [1934] for the control of loose smut [*Ustilago tritici* (Pers.) Jens.], previous experiments were repeated during 1934-35 at the Botanical Sub-Station, Karnal. The variety of wheat tested was Pusa 114 which showed a fair amount of bunt at Karnal during 1934.

The question of soil infection as a factor in the transmission of bunt was later taken up when fully effective results could not be obtained by treating the seed as explained before. In the previous paper the writer mentioned that infection of wheat by bunt (*Tilletia indica*) can take place from soil infested with bunt spores. During 1934-35 further proof was obtained regarding bunt infection through soil. In May 1933 certain plots at Karnal showed ten to fifteen per cent infection of wheat by bunt. Eighteen months later wheat seed which had received treatment with a fungicide was sown in these and other plots, and bunt developed only in those plots where it had previously occurred while other plots were practically free from it.

The fact that infection may be soil-borne as well as seed-borne has been observed by several workers for the other two species of *Tilletia*, [*T. foetens* (Berk. and Curt.) Trel. and *T. caries* (DC.) Tul.] which occur on wheat in various parts of the world. According to Hurst [1935] the ability of the bunt spores to infect the next crop depends largely on conditions of soil temperature, soil moisture and farming practice. He also states that soil infection is a serious factor only in limited areas in the U. S. A. chiefly in the Pacific North-West. Hanna and Popp [1933, 1934] also observed bunt infection of spring wheat by soil-borne spores and state that when bunt spores of *T. tritici* (*T. caries*) in infected heads of Mindum wheat are placed on the surface of the ground, they overwinter successfully in Western Canada. On the other hand Melchers [1934] states that soil infection by bunt spore does not occur in Kansas and that is the reason why bunt has not become a more serious limiting factor in that state. Further Vilkaitis [1933] states that seed treatment with mercurial dust reduces infection in artificially contaminated soil to only a certain extent.

II. EFFECT OF BUNT (*Tilletia indica*) ON WHEAT

Fifty bunt-affected ears of Pusa 165 wheat were collected at random at Karnal in April 1935 and the length of ears and the number of spikelets in each ear were noted and compared with fifty normal ears which were also collected at random. The length and the number of spikelets in healthy and infected ears are shown in Table I together with the number of diseased spikelets in each infected ear.

TABLE I

The length and the number of spikelets in healthy and infected ears and also the number of infected spikelets in infected ears of Pusa 165 during 1935.

No.	Diseased ears			Healthy ears	
	Length (cm.)	Total No. of spikelets	No. of bunted spikelets	Length (cm.)	Total No. of spikelets
1	9·00	14	14	11	17
2	10·5	17	4	14	22
3	10·5	18	8	12·75	19
4	10	19	5	12·5	19
5	10	17	7	11·5	18
6	12	20	1	11·5	18
7	10·5	16	10	12	20
8	10·5	18	2	12	18
9	10	18	7	14	18
10	9	15	2	13	17
11	9	15	8	13	18
12	9	16	4	12	16
13	9·5	16	4	13	19
14	11·5	18	6	12	20
15	10	18	3	10	18
16	9	13	2	11	18
17	11·5	19	1	10	18
18	7	10	1	11·5	19
19	9	16	2	12	18
20	9·5	14	4	11	17
21	10·5	17	1	10	18
22	9·5	16	4	11·75	20
23	10·5	16	3	13·5	21
24	9·75	15	2	14	24
25	10·5	17	2	11·75	20
26	7·75	15	2	10	18
27	8·5	17	4	10	20
28	9	18	6	9	16
29	8	14	14	10	20
30	6	14	14	10	16
31	11	15	1	10	17
32	10·5	17	1	8·5	14
33	10	16	2	12	19
34	10·5	18	4	13·25	19
35	11·25	17	1	14·5	22
36	10·25	20	1	10·5	20
37	10·25	17	2	10·5	18
38	10·5	17	1	13	18
39	10·5	17	4	11·75	19

TABLE I—*contd.*

No.	Diseased ears			Healthy ears	
	Length (cm.)	Total No. of spikelets	No. of bunted spikelets	Length (cm.)	Total No. of spikelets
40	10	16	3	10·75	18
41	11·5	19	2	10·25	17
42	10·25	14	2	12	17
43	9·5	15	1	12	17
44	11	19	4	13·75	20
45	10·25	18	4	14	21
46	10	17	1	10·5	18
47	10	17	3	11	20
48	12	18	5	13	19
49	10·5	18	3	11	17
50	10·5	15	2	12	18
Average	9·94±0·16	16·50±0·29	3·88 ±0·49	11·68± 0·15	18·56± 0·25

From the table it will be seen that the infected plant showed in the majority of cases a reduction in the length of ear and the number of spikelets produced. The mean length of a normal ear and the number of spikelets produced was 11·68 ± 0·15 and 18·56 ± 0·25 respectively as against 9·94 ± 0·16 and 16·50 ± 0·29 in the case of diseased ear.

The ratio of mean difference in length of healthy and infected ear to the standard error of the difference is 7·22. Therefore the difference in length is significant.

The ratio of mean difference in the number of spikelets in healthy and diseased ear to the standard error of the difference is 5·41. Therefore the difference in number of spikelets is also significant.

The number of observations recorded in Table I is rather small for rigid statistical treatment, and during April 1936 a similar comparison was made of 309 ears of Pusa 80-5, 433 of P 165, 128 of Punjab 8A and 132 of Cawnpore 13. The data collected is given in Tables II, III and IV.

TABLE II

Variations and constants for length of healthy and diseased wheat ears

Wheat variety	Healthy (H), Diseased (D)	Frequency classes (cm.)												Total population	Means	Standard deviation	Mode	Ratio of mean difference in length to S.E.	Significant
		3·5	4·5	5·5	6·5	7·5	8·5	9·5	10·5	11·5	12·5	13·5	14·5						
P 80-5	H	..	1	3	18	52	100	69	45	13	7	1	..	309	8·92 ± 0·080	1·41	8·5	6·6	+
	D	1	7	19	39	85	71	50	25	10	2	..	..	309	8·12 ± 0·089	1·58	7·5		
P 165	H	1	2	5	14	34	63	102	119	66	21	5	1	433	9·800 ± 0·078	1·61	10·5	3·9	+
	D	..	2	1	22	54	71	143	88	43	7	2	..	433	9·370 ± 0·069	1·44	9·5		
Punjab 8A	H	..	..	8	39	88	59	22	1	1	..	..	..	218	7·75 ± 0·069	1·03	7·5	3·1	+
	D	4	7	21	54	59	47	18	6	2	..	..	..	218	7·38 ± 0·097	1·44	7·5	—	
Cawnpore 13	H	..	..	1	7	37	43	38	4	2	..	..	..	132	8·48 ± 0·091	1·05	8·5	5·03	+
	D	..	4	7	31	40	27	13	8	2	..	..	..	132	7·71 ± 0·124	1·43	7·5		

+ = Significant.

TABLE III

Variations and constants for the number of spikelets in healthy and bunted ears of wheat

Wheat variety	Healthy (H), Diseased (D)	Frequency classes in number																			Total population	Means	Mode	Standard deviation	Ratio of mean difference to S. E.	Significant
		6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24						
P 80-5	H	...	...	...	...	...	...	6	2	26	10	61	25	86	19	44	17	11	2	...	309	17·62±0·128	18	2·25	5·4	+
	D	...	...	1	0	11	0	18	5	45	3	86	10	69	3	45	4	8	0	1	309	16·51±0·157	16	2·77		
P 165	H	2	1	3	1	7	9	27	30	46	44	50	106	42	24	20	18	2	1	...	433	15·930±0·132	17	2·76	3·4	+
	D	2	0	1	0	18	3	35	12	99	25	120	45	40	12	15	3	3	...	...	433	15·316±0·118	16	2·46		
Punjab 8A	H	...	...	...	...	...	1	6	12	14	38	31	47	33	18	13	3	2	...	...	218	16·52±0·142	17	2·09	6·3	+
	D	1	0	5	0	14	0	21	2	65	...	56	4	34	2	11	1	2	...	...	218	15·01±0·191	14	2·83		
Cawnpore 13	H	...	...	...	...	...	1	0	1	1	19	8	48	5	29	2	18	4	1	...	132	17·85±0·186	17	2·14	9·8	+
	D	...	...	...	2	9	1	14	5	33	9	26	14	8	5	2	2	1	1	...	132	14·91±0·234	14	2·69		

+ = Significant.

In Table II the variations and constants for length of healthy and infected ears of four above-mentioned wheat varieties are shown. In all cases it will be noticed that the mean length of healthy ear is greater than that of bunted ear. The ratio of mean difference in length of healthy and infected ear to the standard error shows that statistically all are significant.

The variations and constants for the number of spikelets in healthy and bunted ears of the four wheat varieties are given in Table III. Here also the mean number of spikelets in healthy ears is greater than that of diseased ears and the differences in number of spikelets in all cases are significant.

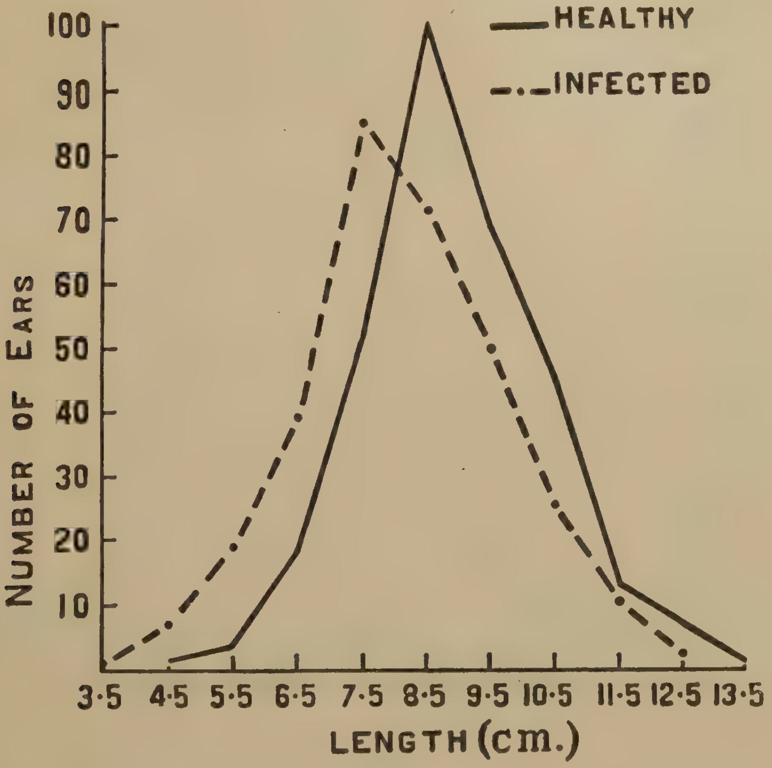


FIG. 1 Comparison of the length of healthy and infected ears of Pusa 80-5.

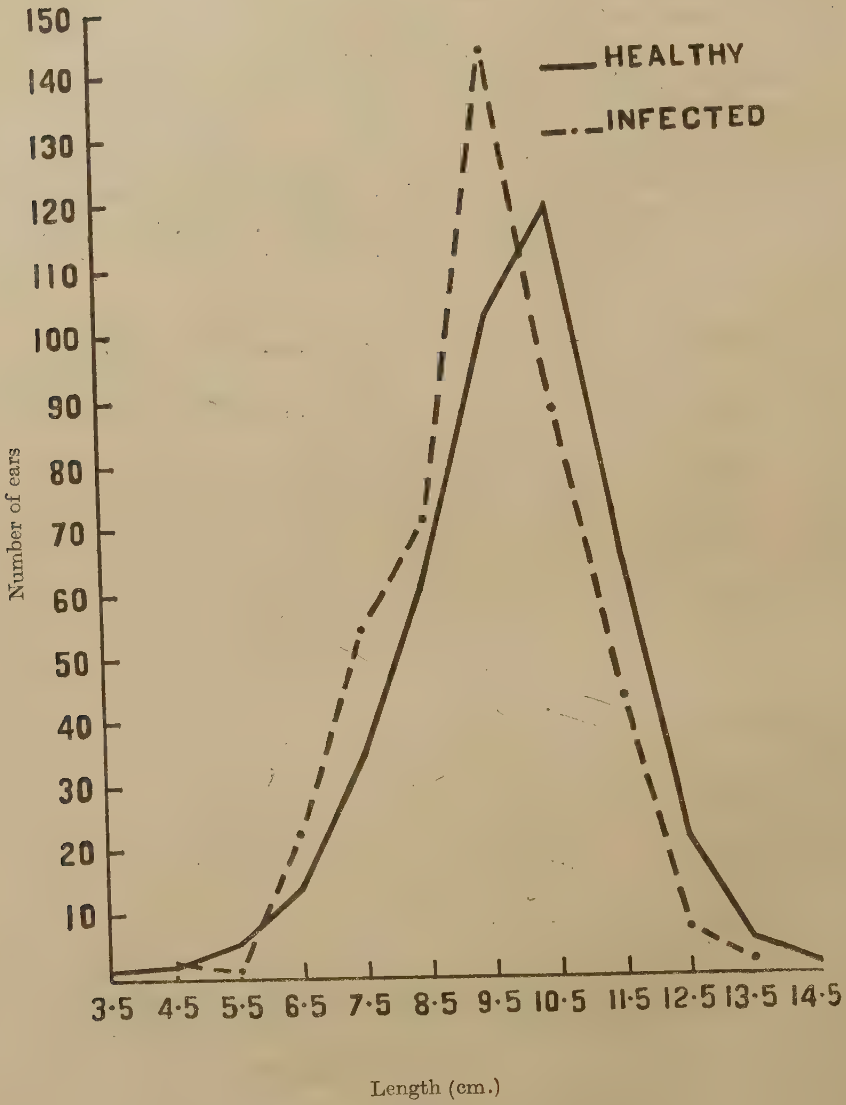


FIG. 2. Comparison of the length of healthy and diseased ears of Pusa 165.

Figs. 1 and 2 represent graphically the difference in the length of healthy and diseased spikelets of P 80-5 and P 165. The observations made during 1936 confirm the conclusions of previous years. Thus like the other two species of *Tilletia* occurring on wheat this species also causes reduction in the length of the ear. Moreover the number of spikelets is also reduced.

Previously [Mitra, 1935] it was observed that a fully infected ear has never been noticed. During 1935, as will be seen from Table I, completely infected ears were found for the first time. During 1936 the number of bunt-affected spikelets per ear was again noted in the four wheat varieties and the data obtained are given in Table IV.

TABLE IV

Variations and constants for the number of bunt-affected spikelets in infected ears of wheat

Wheat variety	Frequency classes in number							Total population	Mean	Standard deviation	Mode
	1	2	3	4	5	6	7				
P 80-5	262	36	9	1	1	..	..	309	1·19±0·029	0·52	1
P 165	368	36	26	3	..	..	..	433	1·22±0·026	0·56	1
Punjab 8A	204	12	2	..	..	..	..	218	1·07±0·019	0·29	1
Cawnpore 13	102	21	4	1	1	1	2	132	1·40±0·088	1·02	1

It will be observed that during 1936 in the majority of cases only one spikelet per ear was found infected even in P 165 where during 1935 fully infected ears were found. The average number of bunt-affected spikelets per ear during 1935 in P 165 was 3·88±0·49 whereas during 1936 it was 1·19±0·029. This shows that the severity of the disease at Karnal was much less in 1936.

III. CONTROL MEASURES BY SEED TREATMENTS

In all the experiments carried out during 1934-35 two types of diseased seed were used :—

Series A.—Naturally infected seed of Pusa 114 was sown.

Series B.—Only heavily infected seed of Pusa 114 as found in nature was picked by hand and sown.

Germination of seed in series A was cent per cent and that in series B eighty per cent under laboratory conditions in Petri dishes.

The treatments were as follows in both the series :—

(*1*) *Control.*—Untreated seed.

(*2*) *Solar energy.*—The seed was soaked in water in a drum from 8 A.M. to noon on a bright day in September 1934 and then spread and exposed to the direct rays of the sun from noon till evening. The seed was then thoroughly dried and stored till sowing time in November.

(*3*) *Sun-heated water.*—The seed was soaked in water heated by solar energy. Water was kept in a blackened drum in the morning under the direct rays of the sun on a bright day in September 1934 and allowed to warm up. At noon the seed was placed in this water and soaked up to 4 P.M. and then removed, thoroughly dried under ordinary conditions by spreading and stored till sowing time in November.

(*4*) *Hot water.*—The seed was soaked in water for four hours, and was later transferred to water at 132°F. for five minutes. The temperature of the water then fell to about 120°-122°F. This seed was again transferred to water at 132°F. for seven minutes. The temperature of this water gradually fell to about 129°-130°F. This hot water treatment was applied to seed just before sowing.

(*5*) *Uspulun.*—The seed was treated for thirty minutes with uspulun (universal) 0·25 per cent solution in water.

(*6*) *Ceresan.*—The seed was treated with ceresan at the rate of two ounces per bushel (60 lbs.)

(*7*) *Granosan.*—The seed was treated with granosan at the rate of two ounces per bushel (60 lbs.)

(*8*) *Copper carbonate.*—The seed was treated with finely powdered copper carbonate at the rate of two ounces per bushel (60 lbs.)

(*9*) *Charcoal formaldehyde.*—The seed was treated with charcoal formaldehyde (fifteen parts of formalin *plus* eighty-five parts of fine charcoal powder).

Treatments (2), (3) and (4) were done according to the methods recommended by Luthra and Sattar [1934].

Treatments (8) and (9) were applied only to seed in series A because of the shortage of seed for series B.

The variations in temperature under the direct rays of the sun as well as that of the water heated by the sun on the dates on which treatments (2) and (3) were accorded, are given below in Table V.

TABLE V

Date	Temperature directly under the sun (°F.)							Temperature of the sun-heated water (°F.)						
	A.M. 8	A.M. 9	A.M. 11	NOON 12	P.M. 2	P.M. 3	P.M. 4	A.M. 8	A.M. 9	A.M. 11	NOON 12	P.M. 2	P.M. 3	P.M. 4
6-9-34 .	112	122	108	120	106	106	96	100	105	108	112	110	110	111

From the above table it will be seen that for treatment (2), the temperature of the water ranged from 100°F. to 112°F. and when the seed was placed under the rays of the sun the temperature was 120°F. but at 4 P.M. it came down to 96°F. For treatment (3) the temperature of the water at noon was 112°F. when the seed was soaked and at 4 P.M. when the seed was removed for drying, it was 111°F.

The experiment reported herein was carried out on a piece of land which was divided into ninety-six small plots, each of which measured 20 feet × 5 feet in area. There were fifty-four such plots in A and forty-two under series B. Each treatment had six replications in both the series of infection. The seed was sown in November 1934 and the crop was harvested next April. At the time of harvesting all the ears in each plot were counted and the number of infected ears was determined. The total number of healthy ears under each treatment and under each replication, for both the series, together with the percentage of bunt is furnished in Table VI.

It is apparent from Table VI that the disinfection of seed by the various treatments employed in this experiment brings about a reduction in the infection of wheat due to bunt (*T. indica*) and that some treatments are more effective in this respect than others. It is also evident that infection in series B in which heavily infected seed was used, was invariably higher than that in series A where the seed had ordinary natural infection. These remarks are confirmed by a statistical study of the data tabulated in Table VI. The analysis of variance of the percentage of infection observed in the experiment is presented in Table VII.

The variance due to treatments is statistically significant in both the series Studying the averages of the percentage infection due to bunt, we find that the various treatments react differently and that the disease is considerably less in plots where seed was treated with ceresan, copper carbonate, uspulun, granosan and hot water in series A and in plots with seed treated with ceresan, granosan, and hot water respectively in series B. Solar energy and sun-heated water do reduce the infection significantly but not to such an extent as the other treatments. This is brought out in Table VIII.

TABLE VI

Number of healthy ears and percentage of infection in Pusa 114 wheat at Karnal in 1934-35

Treatment	Replication I				Replication II				Replication III			
	A		B		A		B		A		B	
	H	Per cent	H	Per cent	H	Per cent	H	Per cent	H	Per cent	H	Per cent
1. Control	2,280	1·31	1,000	2·00	2,180	1·14	840	3·80	2,240	0·93	720	3·47
2. Solar energy	3,518	0·59	1,887	0·63	3,500	0·97	1,798	0·77	3,362	1·01	1,500	0·93
3. Sun-heated water	3,240	1·35	1,326	1·28	3,000	0·83	1,715	0·69	2,600	1·30	1,542	0·64
4. Hot water	2,213	0·36	678	0·50	3,025	0·66	629	0·31	1,760	0·79	600	0·33
5. Uspulun	2,550	0·15	1,351	0·59	2,478	0·36	990	0·60	2,340	0·42	1,025	1·27
6. Ceresan	2,000	0·20	1,060	0·09	2,165	0·13	932	0·10	2,100	0·28	1,310	0·15
7. Granosan	1,900	0·21	850	0·47	1,675	0·23	1,005	0·19	2,000	0·35	1,267	0·15
8. Copper carbonate	1,721	0·34	...	...	1,995	0·45	...	...	2,340	0·25	...	...
9. Charcoal formaldehyde	1,900	0·31	...	...	1,780	0·28	...	...	1,640	0·79	...	...
Total percentage infection		4·17		5·56		4·32		6·46		5·14		6·94
Replication total A and B	9·73				10·78				12·08			

Treatment	Replication IV				Replication V				Replication VI				Treatment total A and B (I to VII)		Total A and B
	A		B		A		B		A		B		A	B	
	H	Per cent	H	Per cent	H	Per cent	H	Per cent	H	Per cent	H	Per cent			
1. Control	2,466	1·33	728	2·33	2,040	1·61	728	2·33	1,810	2·59	660	3·63	8·91	17·56	26·46
2. Solar energy	3,125	0·83	1,852	0·70	2,630	1·06	1,640	0·73	2,957	1·08	1,750	0·68	5·54	4·44	9·98
3. Sun-heated water	2,981	0·63	1,115	0·89	3,424	1·10	805	1·98	2,903	0·86	1,350	0·66	6·07	6·14	12·21
4. Hot water	2,332	0·42	704	0·71	1,950	0·87	510	0·39	1,880	0·15	580	0·51	3·25	2·75	6·00
5. Uspulun	2,615	0·34	628	0·79	2,310	0·25	812	1·72	2,070	0·14	940	1·17	1·66	6·14	7·80
6. Ceresan	1,700	0·05	816	0·24	2,300	0·08	854	0·23	1,600	0·12	926	0·32	0·86	1·30	1·99
7. Granosan	1,850	0·10	1,021	0·39	2,383	0·43	1,040	0·28	1,660	0·18	966	0·72	1·50	2·20	3·70
8. Copper carbonate	1,726	0·40	...	...	2,355	0·17	...	...	1,722	0·17	...	...	1·78	...	...
9. Charcoal formaldehyde	1,972	0·35	...	...	1,730	0·49	...	...	1,621	0·30	...	...	2·52	...	...
Total percentage infection		8·70		6·05		5·40		7·66		5·12		7·69	29·86	40·36	68·25
Replication total A and B	9·75				13·06				12·81				68·25		68·25

H = total number of healthy ears ; per cent = percentage of infection ; A = series A ; B = series B.

TABLE VII

Analysis of variance

Due to	Series A (Ordinary natural infection)					Series B (Heavy infection)				
	Degrees of freedom	Sum of squares	Mean squares	Ratio of S^2_1/S^2_2		Degrees of freedom	Sum of squares	Mean squares	Ratio of S^2_1/S^2_2	
				Observed	Expected at one per cent level				Observed	Expected at one per cent level
Blocks .	5	0·2595	0·0519			5	0·5335	0·1067	..	
Treatments .	8	9·7221	1·2153	16·997	2·99	6	30·7403	5·1234	28·479	3·47
Residual .	40	2·8605	0·0715	..	..	30	5·3956	0·1799	..	
Total .	53	12·8422	..	..	..	41	36·6694	..	..	

TABLE VIII

Average percentage infection of bunt in Pusa 114 wheat treated by various methods at Karnal, 1934-35

Series	Percentage infection	Treatments									General mean	Standard error of mean	Critical difference at	
		(1) Control	(2) Solar energy	(3) Sun-heated water	(4) Hot water	(5) Uspulun	(6) Ceresan	(7) Granosan	(8) Copper carbonate	(9) Charcoal formaldehyde			One per cent	Five per cent
A	Per treatment (average)	1·48	0·92	1·01	0·54	0·28	0·14	0·30	0·25	0·42	0·59	0·1092	0·4714	0·3120
	As a percentage of the control.	100	62·16	68·24	36·49	18·92	9·46	20·27	16·89	28·38	39·87	7·3784	28·0927	...
B	Per treatment (average)	2·93	0·74	1·02	0·46	1·02	0·18	0·37	...	...	0·96	0·1673	0·6734	0·5001
	As a percentage of the control.	100	25·26	34·81	15·70	34·81	6·14	12·63	...	...	32·76	5·7069	22·9289	...

The average percentage of infection due to bunt, therefore, leads to the following conclusions :—

In series A the treated plots were superior to the control. Disinfection with hot water, uspulun, ceresan, granosan, copper carbonate and charcoal formaldehyde is more effective than the two sun treatments. At the five per cent level, treatment with hot water is intermediate between ceresan and the sun treatments.

In series B again all the treatments were effective, ceresan, granosan and hot water being the best.

IV. SOIL INFECTION AS A FACTOR IN THE TRANSMISSION OF BUNT AND TREATMENT

Experiments were carried out at Karnal during 1935-36 (1) to confirm the existence of soil-borne infection by *T. indica*, and (2) to see to what extent this could be controlled by seed treatments. Three varieties of wheat were selected and every precaution was taken to ensure their freedom from bunt infection.

Seed of Pusa 80-5 was obtained from Pusa as bunt does not occur there. Seed of Punjab 8-A was obtained from Lyallpur and was certified as disease-free. Seed of Cawnpore 13 was secured from Cawnpore. Bunt has so far not been recorded from this locality.

The experiment was carried out in a one-third acre field which had heavy infection the previous year. It was divided into six blocks. Each block was further divided into eighteen plots, each plot being 18 ft. × 5 ft. and had five rows one foot apart.

There were altogether six treatments and each block had three varieties and six treatments. Each treatment had six replications.

The following were the treatments :—

(1) Control—No treatment.
(2) Seed treated with ceresan.
(3) ,, ,, ,, agrosan G.
(4) ,, ,, ,, hortisan A.
(5) ,, ,, ,, sulphur.
(6) ,, ,, ,, malachite green.

One pound seed was used for each treatment and for its six replications. The treatments with mercury compounds were given at the rate of two ounces per bushel (60 lbs. of seed) *i.e.*, about one gram of mercury compound for one pound of wheat seed. For treatment (5) *i.e.*, sulphur 3½ gram was used for one pound seed, *i.e.*, at the rate of seven ounces per bushel, and for treatment (6) 500 c.c. of a 1 : 10,000 aqueous solution of malachite green was used per pound of seed, the seed being soaked for four hours and then dried.

At the time of harvesting the healthy and bunt-affected ears from each plot were counted and the percentage of infection was determined. The details of all data collected for all treatments and replications are given in Table IX and the mean percentage of infection for all treatments is given in Table X.

TABLE IX

Number of healthy ears and percentage of bunt

Treatment	Wheat variety	Replication I		Replication II		Replication III		Replication IV		Replication V		Replication VI	
		No. of ears	Per-centage ears diseased	No. of ears	Per-centage ears diseased	No. of ears	Per-centage ears diseased	No. of ears	Per-centage ears diseased	No. of ears	Per-centage ears diseased	No. of ears	Per-centage ears diseased
(1) Control	80-5	1,819	4·06	2,400	2·29	2,131	2·38	2,397	1·71	2,200	3·22	2,250	1·06
	8A	2,600	1·19	3,312	0·60	3,517	0·36	3,730	1·44	3,800	1·15	2,825	0·75
	C13	1,857	1·07	2,380	1·21	2,062	1·26	2,672	1·04	2,540	1·10	2,013	0·24
(2) Ceresan	80-5	2,030	1·03	1,700	1·94	2,414	1·23	3,014	1·32	3,000	1·20	2,450	0·61
	8A	3,211	1·39	3,400	0·61	4,080	0·51	4,266	0·58	3,950	0·75	3,600	0·66
	C13	2,060	0·72	2,100	0·52	2,300	0·43	2,500	0·48	2,300	0·39	2,000	0·45
(3) Agrosan G	80-5	2,900	0·58	2,000	0·49	2,450	0·89	2,200	0·36	2,588	0·46	2,211	0·45
	8A	4,400	0·90	2,780	0·57	3,600	0·47	4,000	0·65	4,288	0·77	2,880	0·69
	C13	3,590	0·27	2,445	0·36	3,480	0·17	3,445	0·43	2,817	0·24	3,280	0·12
(4) Hortisan A	80-5	4,300	0·46	3,800	0·84	3,040	0·29	1,500	0·73	2,518	0·75	1,800	0·44
	8A	3,600	0·25	3,800	0·23	3,300	0·87	4,500	0·35	4,135	0·38	5,056	0·15
	C13	2,500	0·60	2,185	0·41	2,020	0·19	2,130	0·47	2,300	0·35	2,840	0·17
(5) Sulphur	80-5	1,525	0·44	1,940	1·23	2,000	1·15	2,800	0·89	2,580	0·27	2,400	0·37
	8A	3,014	0·49	2,964	0·17	3,200	0·28	2,980	1·07	3,000	0·36	3,800	0·23
	C13	1,666	0·42	2,500	0·24	1,412	0·63	3,600	0·19	2,040	0·34	2,100	0·38
6) Malachite green	80-5	1,300	2·53	1,612	0·99	1,870	0·85	1,925	0·83	2,216	0·63	2,130	0·98
	8A	4,020	0·12	3,368	0·14	3,040	0·20	4,780	0·27	3,800	0·26	4,600	0·10
	C13	1,650	0·30	1,930	0·72	1,600	0·56	2,300	0·56	2,042	0·90	1,900	0·21

TABLE X

Mean percentage of infection for all treatments

Treatments	Wheat varieties		
	P 80-5	Punjab 8A	Cawnpore 13
(1) Control	2·45	0·92	0·99
(2) Ceresan	1·22	0·75	0·50
(3) Agrosan G	0·54	0·68	0·27
(4) Hortisan A	0·59	0·37	0·37
(5) Sulphur	0·73	0·43	0·37
(6) Malachite green	1·14	0·18	0·54

From the data given in the above tables it will be noticed that when disease-free seed is sown in soil in which wheat had shown a good deal of infection in the previous year, bunt makes its appearance to a certain extent. In a previous paper [Mitra, 1935] the writer has mentioned that when disease-free seed is sown in non-infected soil bunt-affected ears are not produced. This proves that soil infection does take place at Karnal and is also responsible for the bunt in wheat.

Further it is noticed that treatments with fungicides before sowing of seed in infected soil reduces the incidence of disease to a certain extent.

The data given in Tables IX and X were statistically analysed and from the analyses of variance it was found that treatments and varietal effects are statistically significant.

Variance . . . . =0·1405
Variance of each mean . . . . . . =0·0236
Standard error for comparison of two means . . =0·217

The conclusion drawn from the statistical analysis is as follows :—

In the case of Pusa 80-5 and Cawnpore 13 agrosan G, hortisan A and sulphur treatments were more effective than the others and in case of Punjab 8A hortisan A, sulphur and malachite green gave better results. Thus agrosan G, hortisan A and sulphur have all given good results. Malachite green has proved to be good only in case of Punjab 8-A. As sulphur is cheaper than the other fungicides, further tests will be carried out with this fungicide.

From the data given in Tables I and II the conclusion drawn is that P 80-5 is more susceptible to bunt than the other two varieties tried and the difference is significant.

As soil infection does take place, crop rotation and growing of resistant wheat varieties is strongly recommended. Further experiments are being carried out to find the minimum period of rotation.

V. EFFECT OF CLIMATIC CONDITION ON BUNT

Previous observations [Mitra, 1935] led to the assumption that bunt does not develop under Pusa conditions. This was further confirmed by observations made during 1934-35. Grains of Pusa 4 and P 165 showing heavy infection with bunt were germinated in sand in a cool incubator and the seedlings were transferred to a plot in Pusa. At the time of harvest there were 1007 ears of P 4 and 1075 of P 165 but none showed any sign of infection. Infected seed of P 4 was also sown in January 1935, *i.e.* when the temperature was low but the plant raised from this seed showed no trace of bunt. Thus under ordinary conditions bunt does not occur at Pusa.

With a view to find out whether bunt would appear if wheat seed is sown in infected soil at Pusa, soil of a heavily infected plot from Karnal was obtained and forty-eight plots were filled and wheat seed sown . At the time of harvest all the ears were counted and examined for bunt. The details are given in Table XI.

TABLE XI

Wheat seed sown in bunt-infected soil at Pusa

Wheat variety	Source of seed	No. of pots	No. of healthy ears	No. of diseased ears	Remarks
P 80-5 .	Infected seed .	8	32	*Nil*	Infected soil from Karnal.
P 114 .	Do. .	8	124	,,	Do.
P 80-5 .	Healthy seed .	8	210	,,	Do.
P 114 .	Do. .	8	103	,,	Do.
P 80-5 .	Infected seed .	8	35	,,	Extra infection given.
P 80-5 .	Healthy seed .	8	118	,,	Do.
P 80-5 .	Infected seed .	8	116	,,	Pusa soil.
P 114 .	Do. .	8	186	,,	Do.

From the above table it will be noticed that bunt does not appear in Pusa even if wheat is sown in infected soil. Wheat was also sown at Karnal on similar soil and bunt was present.

VI. Conclusion

The observations made show that like the other two species of *Tilletia* occurring on wheat, *T. indica* also causes reduction in the length of the ear as well as in the number of spikelets in bunted ears.

The experiments carried out at Karnal during 1934-35 to find out the effect of some of the fungicides, hot water, solar energy and sun-heated water on the elimination of infection by *Tilletia indica* show that the percentage of infection can be substantially reduced by treatment of wheat seed but the disease cannot be completely checked by this means. Thus the previous conclusions [Mitra, 1935] were confirmed.

The experiments carried out during 1935-36 show that bunt infection can take place, to a certain extent, if healthy seed is sown in infected soil. Further the use of certain fungicides reduces the incidence of the disease even if the seed is sown in infected soil.

As soil infection does take place, crop rotation is strongly recommended. It has been further observed that bunt does not appear in Pusa even if wheat seed is sown on infected soil.

VII. Summary

Tilletia indica Mitra causes reduction in the length of the ears as well as in the number of spikelets of the bunted ears.

The effect of some of the fungicides, hot water, solar energy and sun-heated water was tried on the elimination of infection by *T. indica*. The experiments carried out show that the percentage of infection can be substantially reduced by treatment of wheat seed but the disease cannot be checked altogether.

That bunt infection can take place to a certain extent if healthy seed is sown in infected soil has been proved by experiments.

The use of fungicides, such as agrosan G., hortisan A and sulphur reduced the incidence of the disease even if the seed is sown in infected soil.

The use of sulphur may be preferred on account of its cheapness and easy availability.

As soil infection does take place crop rotation is strongly recommended. Experiments are being carried out to find the minimum effective period of rotation.

My thanks are due to Mr. P. V. Krishna Iyer, M.A., Statistician of the Institute, for analysing the data statistically.

REFERENCES

Hanna, W. F. and Popp, W. (1933). *Sci. Agric.* **13**, 636.

——————————— (1934). *Sci. Agric.* **14**, 257.

Harwood, P. M. (1892). *Mich. Agric. Expt. Stn. Bull.* **87**.

Hurst, W. M. (1935). *U. S. Dept. Agric.* (*Washington D C.*) *Cir. Bull.* **361**.

Luthra, J. C. and Sattar, A. (1934). *Ind. Jour. Agric. Sci.* **4**, 177-199.

McAlpine, D. (1910). " The smuts of Australia ", Melbourne.

Melchers, L. E. (1934). *Phytopath.* **24**, 1203.

Mitra, M. (1935). *Ind. Jour. Agric. Sci.* **5**, 51-74.

Potter, A. A. and Coons, G. W. (1918). *Phytopath.* **8**, 106-113.

Rodenhiser, H. A. (1931). *Jour. Agric. Res.* **43**, 465-468.

Sampson, K. and Davis, D. W. (1927). *Ann. App. Biol.* **14**, 86.

Selby, A. D. (1898). *Ohio Agric. Expt. Stn. Bull.* **97**, 31-43.

Vilkaitis, V. (1933). *Dotnava, Lithuamia Metrastis*, p. 69 (Abst. seen in *Expt. Stn. Rec.* **5**, 329, 1935).

THE EFFECT OF DIFFERENT CATIONS ON THE LIVING PROTOPLASM OF THE ROOT HAIR OF *AZOLLA PINNATA**

BY

B. SEN

Vivekananda Laboratory, Almora, U. P.

(Received for publication on 6th February 1937)

(With Plates XXXVIII and XXXIX and one text-figure)

ONE of the chief distinctions of living protoplasm is its complex integrated structure. By structure of protoplasm we do not mean its static components, like parts of a machine, but the distribution of the physically stabler components. Part of the protoplasmic structure is visible microscopically. Beyond the visible structure is postulated an 'ultimate' structure of a quite different character, to which are ascribed the more fundamental properties of protoplasm. Of the nature of the ultimate structure very little is known, and therefore it is a source of many conflicting speculations [Heilbrunn, 1928 ; Seifriz, 1936].

It is with the visible structure that my studies are concerned. Living protoplasm has to be studied in its natural container, which is a microscopic cell. The chemist has the advantage that he can choose his convenient container. Protoplasm being a complex colloid, still imperfectly understood, its structure is best revealed under the ultra-microscope, or dark-field illumination. Cells stabilised by fixation and artificially differentiated by staining cannot offer any reliable guide to the structure of the living protoplasm, as Hardy and Fischer have shown. After all, the difference between a living and a fixed protoplasm is practically the difference between the quick and the dead.

Therefore one of the chief problems in investigating the properties of living protoplasm is to discover a cell which will contain a good supply of protoplasm without colouring matter, without any autonomous movement of the cell as a whole or of its protoplasmic content, a cell easily available throughout the year and which can be kept alive for at least a day or two for observation under the high power of the ultra-microscope. The use of the ultra-microscope introduces further complications, as anyone who has worked with an ultra-microscope will realise. The cell has to be either a unicellular organism, or a tissue one cell thick. The outer membrane of the cell, or the cell-wall, in the case of plants, must be optically homogeneous. The diameter of the cell must not be so small that the defraction image of the wall interferes with the observation of the cell content, and the cell must not contain a large number of bodies which scatter light.

---

*Paper read at the 1937 session of the Indian Science Congress. Published with permission.

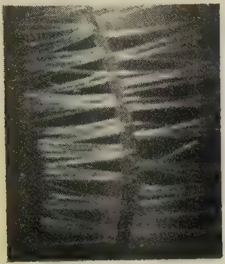
The root hair of *Azolla pinnata*, which I first accidentally discovered in my supply of *Nitella*, seems to fulfil all these specifications. Incidentally, all young root hairs, always single cells, not only contain a generous supply of obliging protoplasm, but they happen to be the cells through which most green plants absorb materials from the soil. The significance of these cells in agriculture is obvious.

The many advantages of *Azolla* are : (*i*) it grows in great abundance throughout the year in all parts of India ; (*ii*) the number of hairs in a single root varies from 400 to 700, and these offer comparable material for several experiments ; (*iii*) root hairs can be easily isolated under water, their natural environment, and can be kept alive for nearly three days under suitable mounting ; (*iv*) the length of individual root hairs varies from 0·5 mm. to 2·5 mm., and the diameter varies from 16 to 25 μ and the hairs are therefore very suitable for observations in the dark-field ; and (*v*) except for a few microns at the tip and at the base, an *Azolla* root hair is practically a cellulose capillary of uniform diameter, filled with transparent protoplasm, in which the only natural movement that can be observed is the Brownian movement of the protoplasmic suspension (Plate XXXVIII, A and B).

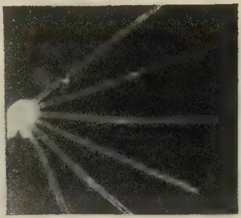
The technique employed for observation of living protoplasm is quite simple. Root hairs are isolated under water and mounted in a hanging drop of water or the solution whose effect is to be observed. A large coverslip is used for the hanging drop preparation. To prevent evaporation of the drop, it is covered with liquid paraffin. This overcomes the use of the moist chamber (Fig. 1). Leitz's cardioid condenser which can be used with micro-manipulators was used for dark-ground illumination. On account of the Brownian movement of the protoplasmic particles of living cells photo-micrographs had to be taken with a motion-picture camera.

(All the figures are dark-field untouched photo-micrographs of root-hair of *Azolla pinnata*.)

(A)

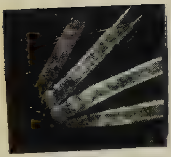


(1) Root with hairs.

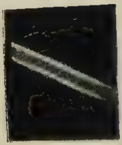


(2) Portion of root with hairs for mounting on cover slip.

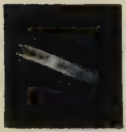
(B)



(1) Base of hairs.

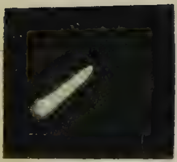


(2) Middle portion of hair.



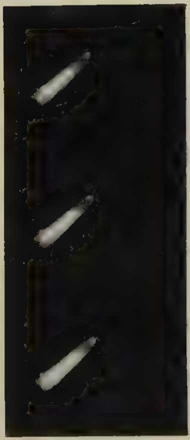
(3) Tip of hair.

(C)



(1) Tip of hair before passing any current.

(2) Same hair subjected to electric current with electrode near tip, anode.



(3) Direction of current reversed with electrode near tip, cathode.

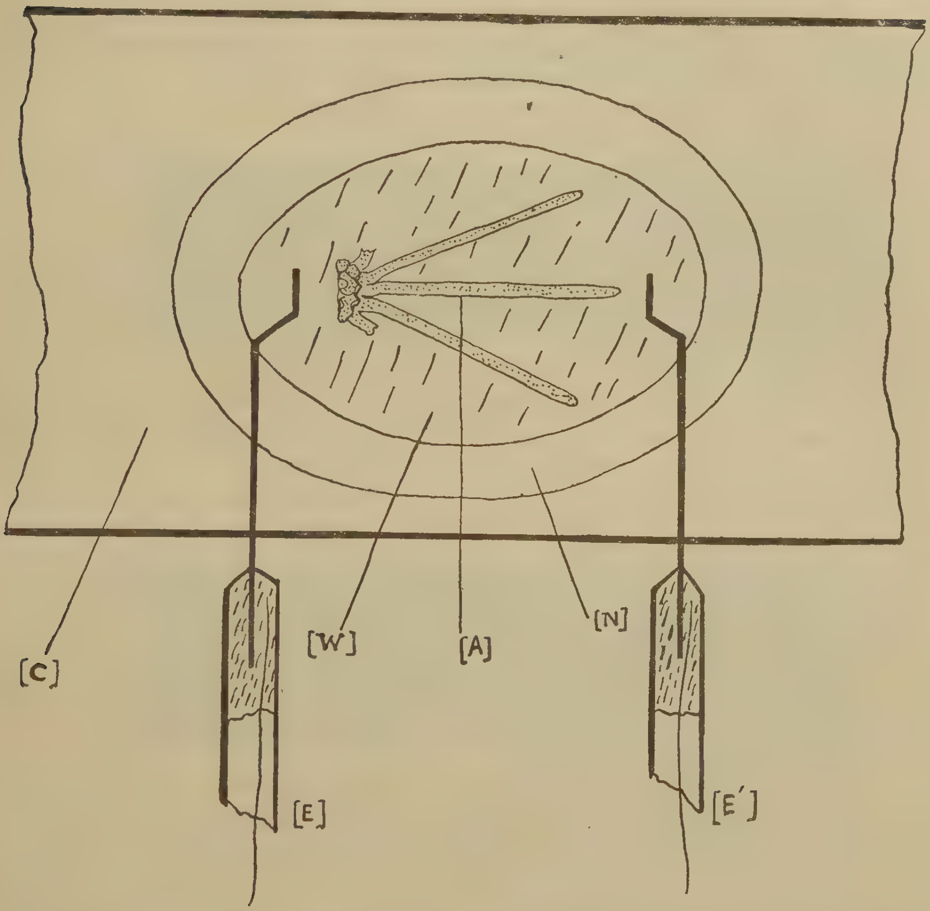


FIG. 1. Diagram of experimental arrangement as seen from above. C, coverslip ; A, *Azolla* root hair ; W, surrounding water or solution ; N, paraffin film ; E, É electrodes.

To determine the electric charge of the colloidal particles of protoplasm of the root hairs, simple micro-electrodes of my own device [Sen, 1930], platinised glass, were used to make the electric contact with the film of water surrounding the hair. The appropriate positions of the electrodes to subject the hair to a uniform field were adjusted with micro-manipulators. The direction and velocity of the protoplasmic particles in the middle portion of the hair when subjected to an electric field were observed. It was found invariably that the particles migrated to that end of the cell which was nearest the anode, thus showing that the particles are negatively charged [Sen , 1934]. A motion-picture film of the cataphoretic migration of the particles has been taken. For illustration, photographs of the tip of the hair (Plate XXXVIII, C) clearly indicate the direction of cataphoretic migration.

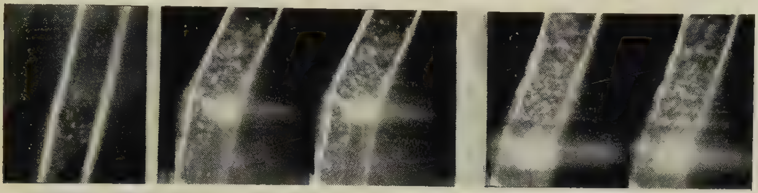
Our knowledge of the effect of different ions on protoplasm is still very meagre, and I have recently been trying to find out the effect of different cations on the negatively charged particles of the *Azolla* root hair protoplasm and whether or not as in the laboratory colloids, the electric charge of the particles can be reversed by the cations of the higher valency.

To study the effect of different ions on living protoplasm, certain difficulties have to be overcome. The very fact that a cell is immersed in a certain iso- or hypotonic solution does not insure that the salt will enter the cell and react with its protoplasm, since the plasma membrane, the outermost layer of protoplasm, is semi-permeable. To overcome this difficulty, the technique of micrurgy has been evolved by Kite, Chambers, Schouton, Peterfi and Taylor [1929]. This technique makes it possible to bring the protoplasm of a cell into direct contact with a solution whose effect is to be observed, either by injecting the solution into the cell, with a micro-pipette operated by micro-manipulators or by puncturing the cell immersed in the solution with a fine micro-needle. I first used the micrurgical technique for my observations. In course of the experiments the limitation of this technique became evident. The direct method of introducing solution into a cell with the finest micro-pipette or puncturing it with a micro-needle entails a mechanical injury comparable, in human terms, to a hypodermic injection with a needle the size of a fire-hose. Moreover, micro-injection cannot be graded or repeated accurately. The same cell cannot be used conveniently for studying the effects of different salts. Another obvious limitation is that one cannot study the effect of salts on a tissue, for it is not possible to inject solutions into all the cells of the tissue.

The ' Increased Permeability ' method which I described at the International Congress of Physiology, held in Leningrad and Moscow in August 1935, appears to solve the above difficulties. The technique is as simple as it is effective. Working with plant tissue, and using its electric conductivity as a measure of the ionic permeability of the plasma membrane of the cells of a tissue, I found that whenever a tissue is effectively stimulated, the permeability of the plasma membrane of the cells to ions increases and that this increase of permeability is, within limits, proportional to the intensity of the stimulus applied [Sen, 1923]. Later, working with single cells of *Nitella*, I was able to confirm the above conclusion [Sen, 1931]. This fact of increased permeability of the plasma membrane to ions I utilised for facilitating the entrance of solution inside the cell. The permeability of the plasma membrane of a cell or a tissue is first increased by an adequate electric shock before the experimental material is immersed in the solution whose effect is to be observed (Plate XXXIX, D). To save time or to observe the immediate effect one can stimulate the cell or tissue immersed in the solution. But when a cell is stimulated in a highly conducting solution most of the current will pass through the solution and not through the cell. Furthermore, when working with single cells in a thin film of solution, the electro-chemical reactions at the electrodes introduce unknown

(All the figures are dark-field untouched photo-micrographs of root-hairs of *Azolla pinnata*.)

(A)

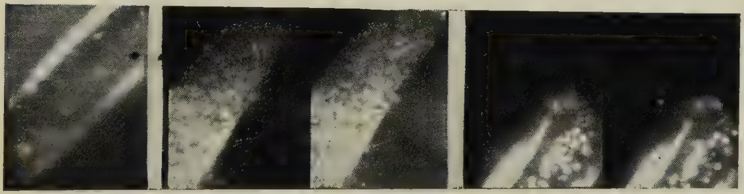


(1) Middle portion of hair, normal.

(2) Immediately after $M/30$ $CaCl_2$ injection.

(3) Two minutes later.

(B)

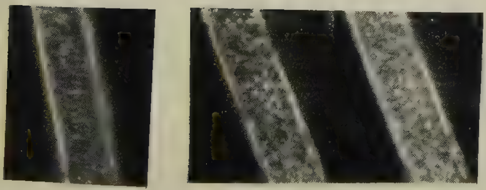


(1) Middle portion of hair mounted in $M/30$ $CaCl_2$ solution, normal.

(2) Immediately after pricking with blunt needle.

(3) Five minutes later, showing formation of larger granules.

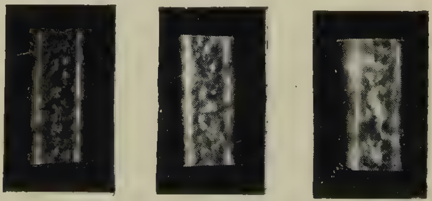
(C)



(1) Middle portion of hair immersed in $M/30$ $CaCl_2$ solution, normal.

(2) Immediately after stimulating hair with induction shock.

(D)



(1) Hair stimulated in water.

(2) Immediately after replacing water with $M/30$ $CaCl_2$.

(3) Five minutes later.

and significant complications, to say nothing of gassing at the electrodes, which is very annoying and interferes with ultra-microscopic observations. This method of increased permeability has the advantage that the electric stimulus, and with it the extent of the increase of permeability, can be graded and repeated accurately. The effect of different salts applied successively can be observed, and thus excellent facilities are afforded for investigation of antagonism of different ions.

Using both micrurgical, and 'Increased Permeability' techniques, effects of chlorides of sodium, potassium, barium, magnesium, strontium, and calcium on the protoplasm of the *Azolla* root hair were observed. $M/20$ solutions of sodium chloride and potassium chloride were used, and $M/30$ solutions of the bivalent cations. Solutions were made with Pyrex glass distilled water. The pH of the solutions varied from 6·8 to 7.

In interpreting the results of observations on living protoplasm, it is well to remember that we do not know what the particles of protoplasm actually are; neither do we possess any definite knowledge of the composition of the dispersion medium of protoplasm. Remembering that one is dealing with entities less than a μ in size and with very thin films, one has to be on guard that one is not observing a series of consistent errors. Realising all these points, one can do no more than list the observed facts and wait for further data before coming to any definite generalisation.

The visible effects of NaCl and KCl.—These were found to be similar. When a hair immersed in a thin film of these solutions is punctured with a micro-needle, the very thin film which forms round the exudate is not stable and often bursts rhythmically. Punctures with a very fine needle (1μ tip) are repaired, but a puncture with a needle of 3-4 μ tip is fatal. The entire protoplasmic content oozes out and the cell dies. When the exudate comes in contact with the surrounding solution, new granules, similar to those found normally in the cell, are formed. Injection of $M/20$ solutions of sodium chloride and potassium chloride into the hair produces new granules and no film forms between the minute injected droplet and the bulk of the protoplasm. When the hair is previously stimulated in water and the film of water replaced with micro-pipette by sodium chloride or potassium chloride solutions, new granules form. The viscosity of the protoplasm, as indicated by the more vigorous Brownian movements of the protoplasmic granules, seems to decrease. The cataphoretic migration of the particles shows that they are negatively charged.

The visible effects of chlorides of Ba and Mg.—$M/30$ solutions of the chlorides of bivalent cations were used. The visible effects of barium chloride and magnesium chloride were found to be similar. When a hair is immersed in solutions of these salts and is punctured with a micro-needle, the film that forms round the exudate is not very stable and the new particles which are formed soon coalesce into an irregular pattern. These salts are very toxic, at least in the concentration used. Barium was found to be more toxic than magnesium. When a solution of these salts is injected into the hair, or when a hair is previously stimulated in water and the surrounding water is then replaced by solutions of these salts, numerous particles are formed which gradually coalesce into an irregular pattern, and the cell dies. Before coagulation of the protoplasm and death of the hair, the electric charge of the particles remains negative.

The visible effects of chlorides of Ca and Sr.—These are similar. When a hair is punctured in solutions of these, the film which forms round the exudate is very stable compared to those formed in barium and magnesium solutions. The puncture is quickly repaired. One can puncture a cell immersed in calcium chloride with a fine micro-needle three or four times and yet the cell will live. Injection experiments show that strontium and calcium produce similar types of granules, mostly spherical. The first-formed small particles coalesce into larger ones. Strontium was found to be more toxic than calcium.

But the most peculiar effect of calcium and strontium is on the film of protoplasm next to the cell wall. When a hair immersed in solutions of either of these salts is punctured, after the first exudate the puncture is easily repaired. But if one moves the needle inserted in the hair to and fro and insures a contact of the surrounding solution with the film next to the wall, a beautiful wave of precipitation starts in both directions from the lacerated portion of the hair, with a velocity of about 2 μ per second. These spherical particles coalesce into larger globules, sometimes of a diameter of about 2 μ (Plate XXXIX, A and B). Even these large particles show Brownian displacements and remain negatively charged, till the protoplasm coagulates and the cell dies.

When a hair previously stimulated is surrounded with calcium chloride or strontium chloride, a shower of particles appears in the protoplasm of the hair. In the dark-field and high magnification it appears like a beautiful snowfall from the cell wall of the hair (Plate XXXIX, C). These particles behave exactly like the granules formed by the injection of these solutions into the hair. In fact, it was this calcium effect which gave me the first visual demonstration of the many possibilities of the 'Increased Permeability' technique.

This effect of calcium is not peculiar to the protoplasm of the *Azolla* root hair. I found that the protoplasm of the giant nerves of *Sepia*, the cuttle fish, with which I worked in Plymouth during the winter of 1935, showed a similar calcium effect.

The effect of calcium on life processes has been a subject of study for numerous investigators, and its rôle in maintaining the normal life functions has long been recognised both by animal and plant physiologists [Farr, 1927]. But the precise nature of its contribution is still very obscure. In plant physiology we have the rôle of calcium in metabolic processes and translocation, and the antagonism which it exhibits towards hydrogen ions, and there is the calcium pectate of the cell wall of the root hairs. The results of the above experiments show that calcium contributes rigidity to the protoplasmic film and that its capacity to repair injury is one of its chief characteristics. The observed similarity of the effects of calcium and strontium on protoplasm also explains the fact why strontium can replace calcium, as has been observed by many physiologists. The effect of calcium and strontium on the protoplasmic film next to the cell wall may offer a reliable idea about the nature of the outermost layer of protoplasm, the plasma membrane. Pending the results of experiments now in progress in my laboratory on the effect of different anions and the antagonism of different ions on the protoplasm of the root hair, it would be premature to make any positive statement. The 'Increased Permeability' technique is also being used to study the effects of different ions on the germination, growth and development of seeds.

Expenses of this investigation have been met by grants from Mr. and Mrs. L. K. Elmhirst and the Imperial Council of Agricultural Research.

SUMMARY

The visible changes induced in the living protoplasm of *Azolla* root hair by isotonic solutions of the chlorides of sodium, potassium (*M*/20), barium, magnesium, strontium and calcium (*M*/30), *p*H varying from 6·8 to 7, have been observed under the high power of the ultra-microscope and photo-micrographs have been taken. The usual micrurgial technique and also my ' Increased Permeability ' technique were used. Both gave similar results. The advantages of the ' Increased Permeability ' technique are described.

Sodium and potassium produce similar visible effects. The new granules formed by these cations are similar to the normal protoplasmic granules. They are negatively charged. The bivalent cations studied, produce larger granules which soon flocculate into an irregular pattern, but no reversal of the electric charge was found to take place. Calcium and strontium of the bivalent cations show similar visible effects in regard to the character of the granules formed and also the mode of formation. When protoplasm comes in contact with solutions of calcium and strontium, a stable film is formed.

REFERENCES

Chambers, R. (1929). ' McClung's Text Book of Microscopical Technique '. New York.

Farr, C. H. (1927). *Amer. Jour. Bot.* **14**, 553-64.

Heilbrunn, L. V. (1928). ' The Colloid Chemistry of Protoplasm. ' Berlin.

Seifriz, W. (1936). ' Protoplasm. ' New York.

Sen, B. (1923). *Proc. Roy. Soc. B* **94**, 216-34.

——— (1930). *Proc. Soc. Expt. Biol. and Med.* **27**, 310-12.

——— (1931). *Ann. Bot.* **45**, 527-31.

——— (1934). *Ann. Bot.* **48**, 143-51.

MOTES IN COTTON

II. PUNJAB *DESI* COTTONS

BY

MOHAMMAD AFZAL

Cotton Research Laboratory, Lyallpur

(Received for publication on 3rd February 1937)

INTRODUCTORY

THE fertilisation of ovules and their development into mature seeds in various crop plants, especially of the tropical regions, is a subject the importance of which is not yet adequately realised. The undeveloped ovules represent a direct loss of the crop to the grower. During a preliminary enquiry on cacao, Pound [1932] found that the number of beans was markedly variable from pod to pod on the same tree whereas the number of ovules was very constant from ovary to ovary. The number of ovules which failed to produce beans was variable and in extreme cases twenty-five to fifty per cent of the ovules did not produce any beans. He was not able to locate the exact cause but says that genetic causes, incomplete pollination and physiological conditions were responsible. In cotton such undeveloped ovules, usually called motes, represent a serious loss not only to the grower but also to the manufacturer as these motes besides failing to produce any fibre of commercial value also cause defects in yarns and offer serious impediments in the way of clean spinning, dyeing and weaving. According to Midgley [1933] there is no way of satisfactorily removing them apart perhaps from combing. A knowledge of the number and position of motes occurring in various commercial varieties of cotton would be of great importance to the breeder, as then alone can he try to remove this defect. With this object in view a detailed enquiry into the number of motes and the factors which bring about their production in the Punjab cottons was started in 1929. The results on Punjab-American cottons have already been published [Afzal and Trought, 1934] and the present communication deals with the Punjab *desi* cottons.

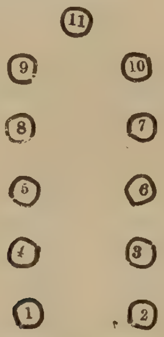
The previous literature on this subject has already been discussed in the first paper of this series and will not be reviewed here. The experimental procedure followed was the same as in the case of American cottons and is only briefly described below :—

In order to find out the ratio of motes to ovules (referred to in the text as percentage of motes), fifty average plants of 10 Rosea (*G. neglectum*, var. *rosea* Gammie), 15 Mollisoni (*G. indicum*, var. *Mollisoni* Gammie) and 12 Sanguineum (*G. sanguineum* Gammie), the three most important strains of Punjab *desi* cottons, were selected at random from the seed multiplication area at the Cotton Research

Farm, Risalewala, during 1931 and 1932. The bolls which matured on these plants were picked separately along with the burr. Pickings were taken after every ten days, thus making a total of six to eight pickings for the various varieties. Dates of pickings in both years were the same and the results are, therefore, strictly comparable.

EXPERIMENTAL

The various pickings of all the three cottons were examined separately with regard to the number of bolls per plant, the number of ovules per lock and the number and position of motes in the lock. The position of motes in the lock was determined, as in the case of American cottons, according to the following method of numbering the ovules in a lock placed with the base of the lock near the observer and its inner surface turned upwards.



The data thus obtained are tabulated below :

TABLE I

Bolls and motes of three varieties of desi *cottons*

—	10 Rosea		12 Sanguineum		15 Mollisoni	
	1931	1932	1931	1932	1931	1932
No. of plants examined.	50	50	50	50	50	50
No. of bolls examined.	488	663	1531	1021	1318	839
Mean No. of locks per boll.	3·13 ±0·0140	3·31 ±0·0196	3·37 ±0.0131	3·58 ±0·0174	3·15 ±0·0099	3·18 ±0·0128
Mean No. of ovules per lock.	8·20 ±0·0272	8·46 ±0·0212	8·02 ±0·0121	7·78 ±0·0140	8·73 ±0·0039	8·88 ±0·0062
Percentage of motes.	6·47 ±0·229	6·42 ±0·191	5·86 ±0·120	7·78 ±0·169	8·95 ±0·156	16·59 ±0·257

The percentage of motes in 15 Mollisoni during 1932 was abnormally high. This was probably due to the very alkaline nature of the land on which the crop was grown. The yield was also below normal.

A comparison of the percentage of motes in various varieties indicate that 15 Mollisoni had a significantly higher percentage than either of the other cottons in both years. The differences between 10 Rosea and 12 Sanguineum were, however, only barely significant in 1931 and significant in 1932.

The values of *t* and *P* are given in Table II.

TABLE II

Value of t *and* P *for the percentage of motes in* desi *cotton*

——	*t*	*P*	Remarks
15 Mollisoni × 12 Sanguineum—			
1931	15·7	<0·01	Significant.
1932	28·7	<0·01	,,
15 Mollisoni × 10 Rosea—			
1931	8·9	<0·01	,,
1932	31·7	<0·01	,,
10 Rosea × 12 Sanguineum—			
1931	2·3	<0·05	Just significant.
1932	5·3	<0·01	Significant.

Another interesting comparison would be between percentage of motes in American and *desi* cottons. The figures for the former varieties have already been reported [Afzal and Trought, 1934] and of the later types in Table I above. The comparison has shown that the value of *P* lies between 0·01 and 0·05 for the observed $t=2{\cdot}534$ as seen against $n=8$ in Fisher's '*t*'-tables [Fisher, 1934]. It can, therefore, be stated that the percentage of motes in Americanc ottons was significantly higher than in the *desi* cottons.

As the different pickings were examined separately, the analysis of the data given in Table III,

TABLE III

Percentage of motes in different pickings

Pickings	10 Rosea		12 Sanguineum		15 Mollisoni	
	1931	1932	1931	1932	1931	1932
1st (6th October) . .	..	9·18	11·61	11·59	23·78	18·26
2nd (21st October) . .	..	9·63	12·10	8·46	17·40	17·17
3rd (5th November) . .	6·13	5·43	9·20	7·75	11·18	14·07
4th (25th November) . .	7·10	5·80	4·48	7·50	9·70	15·38
5th (5th December) . .	5·89	6·97	4·11	6·65	8·90	10·07
6th (20th December) . .	6·56	6·20	4·30	7·54	7·26	14·19
7th (4th January) . .	7·17	6·40	5·70	6·65	9·0	18·10
8th (25th January) . .	7·68	16·60	5·74	..	9·25	..

The figures in Table III were analysed statistically as shown in Table IV.

TABLE IV

Analysis of variance and co-variance (motes/ovules)

——	Degrees of freedom	Sum of squares : Σy^2 (Motes)	Sum of squares : Σx^2 (Ovules)	Sum of products Σxy
Between pickings . .	7	10059·4	508328·9	52415·6
Between varieties . .	2	5967·8	323277·4	28627·4
Error	33	36274·7	2750237·7	270038·0
Total .	42	52301·9	3581844·0	351081·0

The adjusted analysis of variance (motes/ovules) is given in Table V. For this analysis the unit was changed to a multiple of ten.

TABLE V

Adjusted analysis of variance

——	Degrees of freedom	Sum of squares	Mean square	Ratio of variances x
Between pickings . .	7	9032·8	1290·4	4·18*xx*
Between varieties . .	2	3422·35	1711·17	5·56*xx*
Error	32†	9859·6	308·1	

† One degree of freedom has been absorbed by the linear regression.

It is evident from Table V that the differences between varieties and pickings are significant.

The early and late pickings had a comparatively greater number of motes than the middle pickings. Cotton picked in the middle of the season, as is well known, is always better in grade and ginning outturn than that picked either early or late and the smaller number of motes in the middle pickings seem fortunate. Or is it that the causes responsible for the better grade and ginning outturn of the middle pickings are also conducive to the production of a lower percentage of motes ? This point will, however, be referred to later.

It may also be mentioned that during both the years not a single plant was found entirely free of motes. Several bolls were, however, met with which had all the ovules properly developed and formed into seeds. Such perfect bolls were counted separately and the figures are set out in Table VI.

TABLE VI

Percentage of perfect bolls in desi *cottons*

Varieties		10 Rosea		12 Sanguineum		15 Mollisoni	
Pickings	Year	1931	1932	1931	1932	1931	1932
I	3-locked . . .	..	21·7	13·3	..	..	4·4
	4-locked . . .	..	16·7	15·8	14·6	*Nil*	*Nil*
II	3-locked . . .	42·6	11·3	11·6	21·5	5·8	3·9
	4-locked . . .	..	18·6	14·8	17·4	10·0	2·5
III	3-locked . . .	33·4	34·4	19·6	30·9	20·6	8·3
	4-locked . . .	31·2	25·4	21·6	22·5	*Nil*	3·0
IV	3-locked . . .	35·8	26·4	45·9	21·5	16·5	12·0
	4-locked . . .	16·7	42·8	26·5	18·4	*Nil*	*Nil*
V	3-locked . . .	39·6	28·3	48·6	35·4	17·9	21·5
	4-locked . . .	33·3	21·2	32·4	35·9	5·2	*Nil*
VI	3-locked . . .	34·8	44·0	48·3	25·0	23·2	7·7
	4-locked . . .	40·0	29·3	36·4	22·6	6·6	*Nil*
VII	3-locked . . .	31·3	41·1	34·8	39·1	14·6	*Nil*
	4-locked . . .	27·3	30·7	27·9	17·0	9·7	*Nil*
VIII	3-locked . . .	27·3	20·0	46·0	..	14·9	*Nil*
	4-locked . . .	*Nil*	*Nil*	33·3	33·3	3·7	*Nil*
Whole season							
	3-locked . . .	36·6	32·4	41·8	30·4	17·6	5·3
	4-locked . . .	29·2	25·7	26·5	20·7	6·4	2·0

A consideration of Table VI will reveal that the number of bolls free of motes was smaller in 1932 than in 1931, especially in the case of 12 Sanguineum and 15 Mollisoni. A feature of special interest, however, is the smaller proportion of perfect bolls when the bolls were 4-locked as compared to the number of such bolls in the case of 3-locked bolls. The total number of perfect 3-locked bolls in the case of all varieties during both the years was 1,075 or 26·2 per cent of the total, while the number of perfect 4-locked bolls was only 352 or 20·19 per cent of the total. No comment is necessary on the figures. The smaller number of perfect bolls with four locks was probably due to the greater competition for nutrients among the developing ovules in these bolls as compared with the 3-locked bolls.

An attempt was made to compare the variations in the percentage of motes in the different bolls of the same plant and the percentage of motes in different plants of the same strain. Such a comparison, besides affording some information regarding the causes of mote production would yield very valuable information on the methods of removing this defect. These figures are given in Table VII.

TABLE VII

Adjusted analysis of variance (motes/ovules)

Varieties and year	Between plants		Between bolls of same plant		Ratio of variances *x*	Remarks
	Degrees of freedom	Mean variance	Degrees of freedom	Mean variance		
10 Rosea—						
1931 . .	49	26·7	319	1·17	22·8*xx*	Significant.
1932 . .	49	39·8	601	0·19	209·0*xx*	,,
12 Sanguineum—						
1931 . .	49	111·4	1479	0·17	655·0*xx*	,,
1932 . .	49	91·4	961	0·22	415·4*xx*	,,
15 Mollisoni—						
1931 . .	49	107·6	1258	0·57	188·7*xx*	,,
1932 . .	49	140·0	773	0·84	166·6*xx*	,,

It is evident from Table VII that the variation in the percentage of motes between plants is significantly greater than the variation between bolls of the same plant. This would mean that the nutritional defect which is the most important factor in causing the production of motes is operative for the whole plant and not in the case of individual bolls. This factor may also have a selective value and the breeder may, by proper selection, be able to mitigate this defect. This phase of the problem, however, requires further work to see whether the percentage of motes is a hereditary character or not. Further work is now in progress to test this point.

POSITION OF MOTES IN THE LOCKS OF COTTON

The position of motes in the locks of cotton was also determined and the figures are given in Table VIII. The position was determined according to the diagram on page 488.

TABLE VIII

Position of motes in the locks of cotton

Seed position in the lock

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
					10 Rosea						
Percentage of motes in—											
1931 . .	9·34	9·77	5·78	6·63	6·42	4·85	5·20	4·69	14·33		
1932 . .	9·42	7·42	7·26	5·82	6·03	5·98	5·20	5·33	5·65	13·32	
					12 Sanguineum						
Percentage of motes in—											
1931 . .	6·27	5·94	5·02	4·62	6·20	5·30	6·16	7·19	7·58		
1932 . .	7·67	5·99	6·94	6·58	8·03	7·69	9·24	10·22	9·65	14·54	33·3
					15 Mollisoni						
Percentage of motes in—											
1931 . .	13·0	11·64	9·05	8·87	6·70	7·91	7·11	7·43	8·83		
1932 . .	26·15	22·07	19·48	16·33	12·27	11·75	11·91	12·02	13·58	14·48	71·43

The tendency for a somewhat lower percentage of motes near the middle of the lock is fairly clear. The same tendency was noticed in the case of American cottons [Afzal and Trought, 1934], but it has not been so far possible to find an adequate explanation for this phenomenon. Porter [1936] studied the position of motes in ten varieties of American cotton and found that the average number of motes per lock was 1·38, and that about one-third of these motes were found at the base of the locks.

DISCUSSION ON THE CAUSES OF MOTE PRODUCTION

Opinion on the probable cause of mote production has so far been divided. Defective nutrition [Eaton, 1931; Rae, 1928], defective pollination [Rae, 1928] and lack of soil moisture [Bailey, 1929] have at various times been suggested as the determining factors. It has, however, been shown in an earlier paper [Afzal and Trought, 1934] that defective nutrition is probably the most important single factor which determines the presence and the proportion of motes in any single plant. Further support of the above idea could be adduced from the figures presented in the present paper.

A comparison of the number of motes in *desi* and American cottons shows that American cottons are leading the *desi* by a very wide margin. The *desi* cottons are indigenous to this place. These have also a much better root system and are more hardy and, therefore, capable of nourishing their bolls much better than the American cottons. For this reason these cottons have never suffered from bad opening of bolls, a defect so very common in the American varieties. The bad opening of bolls is a manifestation of malnutrition and the smaller number of motes in the case of *desi* cottons can thus be easily explained on the score of better nutrition. It may also be pointed out that during the flowering season of cotton at Lyallpur, insects are in great abundance and there has never been found a single stigma of an opened flower which did not have its more than due share of pollen in the afternoon.

This fact alone would score out the possibility of defective pollination as a possible cause of mote production. As regards defective fertilisation as a source of mote production evidence has been sought from microscopic studies of motes. It has been observed that all the motes so far examined have shown unmistakable signs of having been fertilised.

Further evidence in support of this idea is forthcoming from a consideration of Table III. The middle pickings in all varieties showed a small number of motes than the early and late pickings. It is but a truism to state that the cotton plant is at its best during this season. There is abundance of food reserve and the climatic conditions are very suitable. The feeding of the bolls therefore goes on unimpaired and the number of motes is consequently reduced. The number of perfect bolls with three locks is more than three times the number of perfect bolls with four locks. The number of ovules in 3- and 4-locked bolls is usually in the proportion of 25 : 33. It is obvious therefore that with a greater number of ovules to feed, the 4-locked bolls will necessarily leave a bigger number ill fed and consequently a higher proportion of motes.

SUMMARY

The number and position of motes in the locks of the three most important *desi* varieties of cotton, namely, 10 Rosea, 12 Sanguineum and 15 Mollisoni were determined during 1931 and 1932.

It was found that the total number of motes in *desi* cottons was far less in comparison with American cotton, but the disposition of the motes in the various seed positions was very similar. The number of motes was least in the centre of the locks.

The early and late pickings had a greater number of motes than the middle pickings.

It has been suggested that by far the most important cause of mote production is the defective nutrition of the developing ovules.

ACKNOWLEDGMENT

My Statistical Assistant, Mr. B. R. Sehgal, is responsible for the statistical examination of the data.

This work was carried out under the Punjab Botanical Research Scheme financed jointly by the Indian Central Cotton Committee and the Punjab Government.

REFERENCES

Afzal, M. and Trought, T. (1934). *Ind. Jour. Agric. Sci.* **4**, 554.

Bailey, M A. (1929). Reports received from Expt. Stations, E. C. G. C., 1928-29, **10**, p. 195.

Eaton, F. M. (1931). *Jour. Agric. Res.* **42**, 455.

Fisher, R. A. (1934). 'Statistical Methods for Research Workers'. Fifth edition (1934) Oliver and Boyd, Edinburgh.

Midgley, E. (1933). Quoted by Pearson, N. L.—*U. S. Dept. Agric. Tech. Bull.* **396**.

Porter, D. D. (1936). *U. S. Dept. Agric. Tech. Bull.* **509**.

Pound, F. J. (1932). *Ann. Report Cacao Research, Imp. Coll. Trop. Agric.* (*Trinidad*).

Rea, H. E. (1923). *Jour. Amer. Soc. Agron.* **20**, No. 10.

THE DEVELOPMENT OF THE MICROSPORES IN *TRICHOSANTHES DIOICA* ROXB.

BY

I. BANERJI

AND

M. C. DAS

Department of Botany, Calcutta University

(Received for publication on 4th February 1937)

(With Plates XL-XLIII and five text-figures)

Trichosanthes dioica is extensively cultivated in Bengal and in the adjoining provinces for its fruits which are much relished as a vegetable. It is a dioecious plant and is mostly propagated by root cuttings.

The literature on the cytology of plants belonging to the family Cucurbitaceæ is very meagre. Kirkwood [1907] worked out the process of pollen formation in *Micrampelis alba* and found that the process of chromosome conjugation was of the parasynaptic type. Strasburger [1910] worked on *Bryonia alba* with an idea to determine the sexual mechanism of the plant. Boenicke [1911] studied the heterotypic cell division in *Bryonia dioica*, and Lundegardh [1914] in *Cucurbita pepo*, *Bryonia alba* and *Bryonia dioica*. Later on, *Bryonia dioica* was re-investigated cytologically by Meurman [1925] and Lindsay [1930]. Kozhuchow's [1925] investigations were mostly confined to somatic cells of a number of Cucurbitaceous plants. He also determined the chromosome numbers of the plants he studied. Castetter [1926] described meiosis in *Cucurbita pepo*. He found that cytokinesis took place by furrowing in this plant. Heimlich [1927] worked out the meiosis in *Cucumis sativa*. Passmore [1930] has given a comprehensive account of the process of pollen formation in *Cucurbita pepo*, *Cucurbita maxima*, *Citrullus vulgaris*, *Luffa cylindrica*, *Cucumis melo* and *Cucumis sativa*. He found that the process of chromosome conjugation was of the parasynaptic type. Asana and Sutaria [1932] have given a very general account of the mode of pollen formation in *Luffa aegyptiaca* and also determined the chromosome number of the plant. Whitaker [1933] has recently determined the chromosome numbers of twelve species of Cucurbitaceous plants and has also made some interspecific crosses.

Sinoto [1928] has observed the presence of a heteromorphic pair of chromosomes in *Trichosanthes japonica*, but Lindsay [1930] and others who worked on *Bryonia dioica* failed to find any heterochromosome. As *Trichosanthes dioica* was not studied cytologically before, it was thought that a cytological study apart from bringing out the essential features of meiosis will also indicate whether a sex chromosome is responsible for the dioecious nature of the plant. With this view the work was primarily undertaken.

From the list of chromosome numbers of Cucurbitaceous plants recently compiled by Asana and Sutaria [1932] it appears that the chromosome number of *Trichosanthes dioica* and *Trichosanthes anguina* had not been previously determined. The latter plant being monoecious, it was thought that a comparative study of the chromosomes of both the plants might throw some light on the sexual mechanism involved. Stages of meiotic division of *Trichosanthes anguina* have, therefore, also been worked out.

MATERIAL AND METHODS

The material for this investigation was obtained from plants grown in the University College gardens. Fixation was done in the field at different hours of the day; best results were obtained from material fixed in the morning between 6 A.M. and 9 A.M., and in the evening between 5-30 P.M. and 7 P.M. The young anthers ranging from 1 to 4 mm. in length were carefully dissected out and immediately transferred to the fixing fluid. A number of fixing fluids were tried, of which Allen's modified Bouin's fluid gave the most satisfactory result. The material was kept in the fixing fluid for twenty-four hours and then dehydrated and cleared in the usual way. After embedding sections were cut six to twelve microns thick.

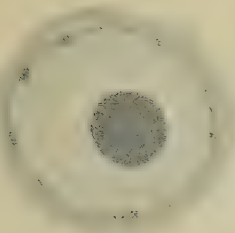
Comparative study of the somatic chromosomes was made from the root tip cells. Adventitious roots obtained from both male and female plants were fixed separately.

Heidenhain's iron alum haematoxylin and Newton's iodine gentian violet stains were chiefly used.

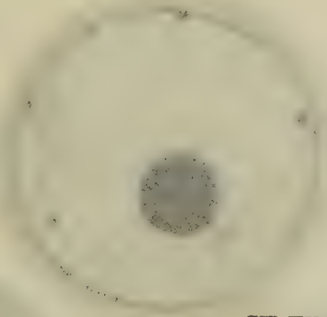
DEVELOPMENT OF THE MICROSPORES

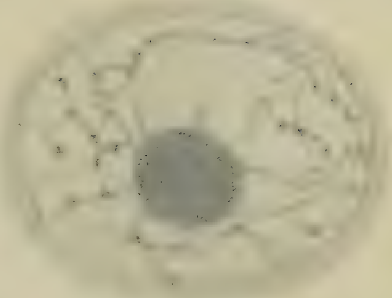
Resting condition of the pollen mother cells.—The pollen mother cells in the resting condition are polygonal in outline. There are no intercellular spaces between them. Each cell is bounded by a very thin wall and is filled with finely granulated cytoplasm. The position of the nucleus is more or less central.

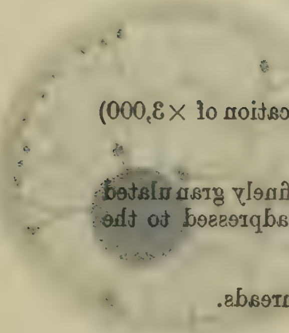
The nucleus of the resting pollen mother cells is spherical in outline and has a well-defined membrane. Within the nuclear membrane and continuous with it is a reticulum composed of finely granulated substance (Plate XL, fig. 1). The position of the nucleolus is more or less central and it is surrounded by a clear zone, which varies in extent from a small to a comparatively large area. The nucleolus lies free in this clear zone and there seems to be no apparent connection with the reticulum. Presence of such a clear zone has been observed in the resting nucleus of a number of plants and there are diverse opinions about it.

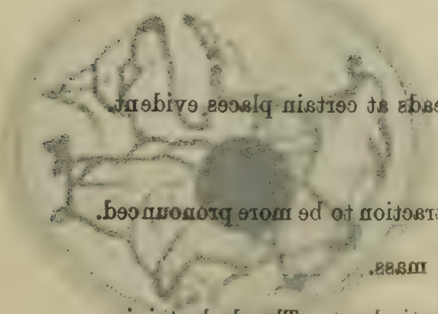


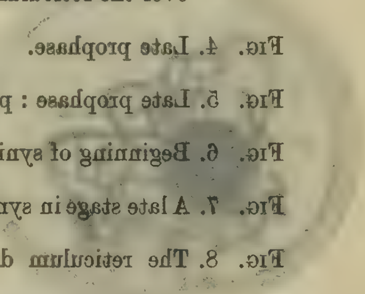
1

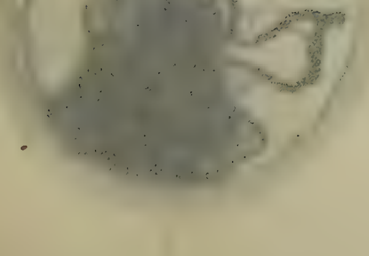












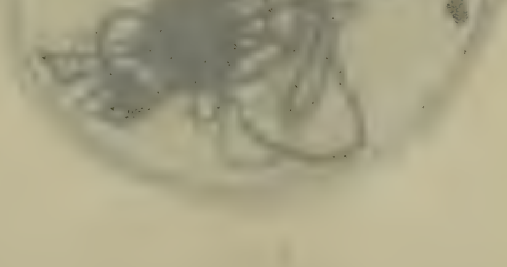


PLATE XL

(All figures were drawn with the aid of a camera lucida at a magnification of ×3,000)

Fig. 1. Resting stage of pollen mother cell : the reticulum has a finely granulated appearance. Dark chromatin bodies are seen closely adpressed to the inner side of the nuclear membrane.

Fig. 2. Early prophase ; the nucleus resolves into definite threads.

Fig. 3. Prophase. The threads have considerably increased in thickness as well as in chromaticity. Note the distribution of chromatin particles over the reticulum.

Fig. 4. Late prophase.

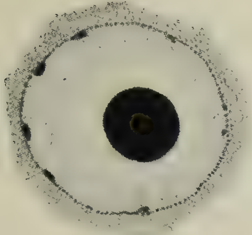
Fig. 5. Late prophase : pairing of leptotene threads at certain places evident.

Fig. 6. Beginning of synizesis.

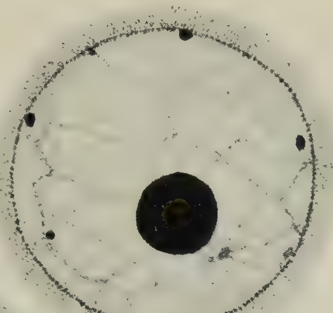
Fig. 7. A late stage in synizesis showing the contraction to be more pronounced.

Fig. 8. The reticulum drawn into a compact mass.

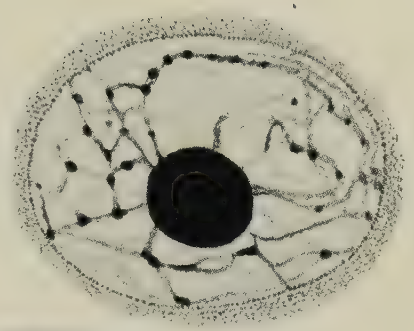
Fig. 9. Loosening of the spireme from the synizetic knot. The dark-staining chromatin bodies noted previously on the reticulum have now formed dark masses which lie on the spireme. These dark bodies are less in number but greater in volume than those noted before.



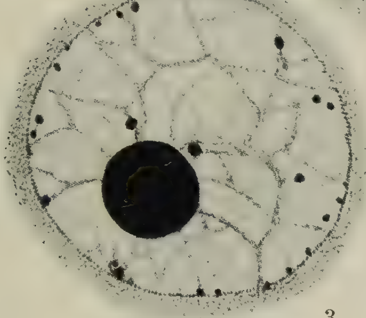
1



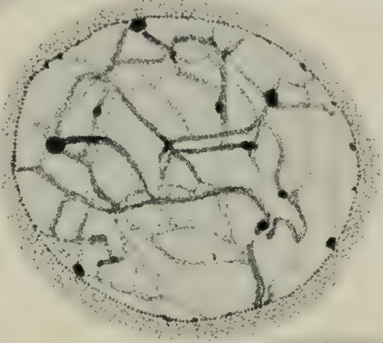
2



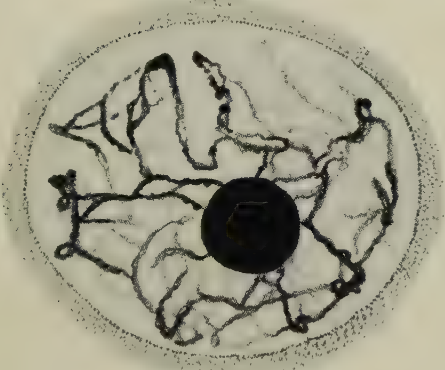
4



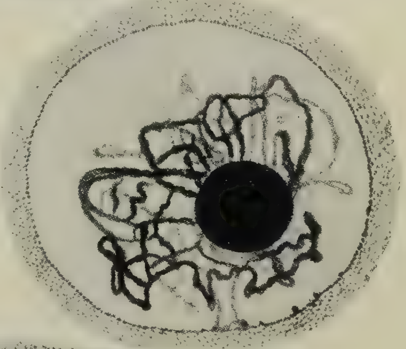
3



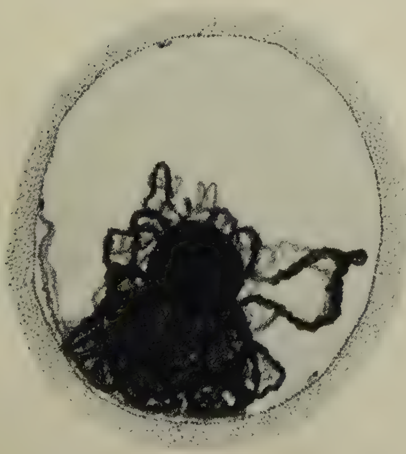
5



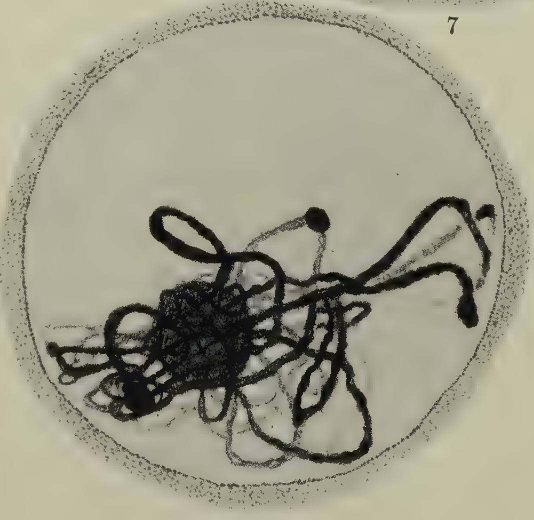
6



7



8



9

Generally one large homogeneous nucleolus is noted inside each nucleus, but in many cells two equal, or one large and one small nucleoli have also been observed.

Some dark-staining bodies closely adhering to the inner membrane of the nucleus are also seen. These are not equal in size and are irregularly arranged on the nuclear membrane. Their exact number is very difficult to determine as they are found to occur irregularly.

Prophase.—With the onset of prophase the nucleus increases in size and simultaneously indications of thread formation are apparent in the reticulum. The fine reticulum gradually spreads over the clear zone outside the nucleolus. The threads composing the reticulum do not appear to be uniformly thick and are very irregularly distributed. The continuity of the threads could not be made out at this stage (Plate XL, fig. 2). The dark-staining bodies noted on the nuclear membrane during the resting condition of the pollen mother cells now appear to have shifted a little towards the centre. Gradually the chromaticity of the threads increases (Plate XL, fig. 3), and indications of lateral pairing of the threads are noted at some places. The dark chromatin bodies which have already shifted centrally are now seen mostly to be scattered on the threads and appear as dark spots. The nucleolus preserves its spherical outline but increases in size. Its position relative to the spireme is also more or less central. Usually it is enclosed by the spireme and is in actual contact with it. Its staining capacity remains the same as before.

During late prophase the threads appear to be uniformly thick and are more or less deeply stained. Close observation of the reticulum at this stage shows that at some places lateral approximation of the threads is taking place. Occasionally two threads approach one another and run parallel for a short distance and then their identity is lost due to anastomoses. Critical examination shows that the leptonema thread is double at places (Plate XL, figs. 4 and 5).

Synizesis.—With the commencement of synizesis the reticulum begins to contract away from the nuclear membrane leaving a clear space round the contracted reticulum (Plate XL, fig. 6). As the contraction proceeds, the association of the threads become more and more prominent. Plate XL, figs. 6 and 7 show the lateral approximation of the threads at several places. As observed before the pairing mates occasionally fuse. Gradually the reticulum is transformed into a tight knot by a process of contraction (Plate XL, fig. 8). The synaptic knot becomes so thick and tight that often it becomes difficult to distinguish the threads therein and it appears as a dark mass. Generally the contracted knot lies at one side of the nuclear cavity enclosing the nucleolus in its meshes. Synizesis seems to be of short duration in this plant. In this stage close association of the synaptic mates is accomplished.

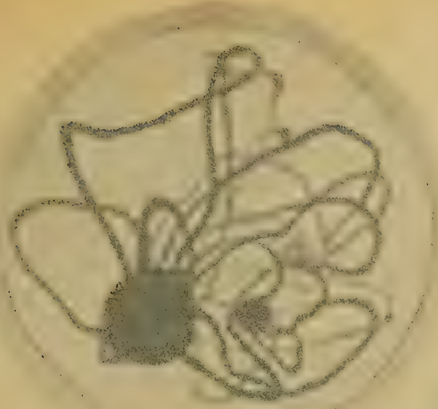
The identity of the dark nuclear bodies which had been noted on the leptotene threads now becomes obscure. This is perhaps due to the twisting and close approximation of the threads during synizesis. A few dark-staining bodies are, however, noted in the cytoplasm at this stage. These cytoplasmic inclusions have also been observed in later stages. Their origin and behaviour during meiosis will be dealt separately elsewhere.

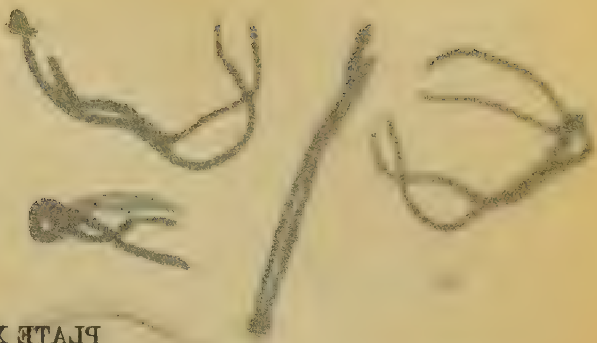
Open spireme.—Shortly after synizesis the knot begins to unravel (Plate XL, fig. 9). It loosens and spreads inside the nuclear cavity (Plate XLI, fig. 10). As the spireme spreads out the threads become more uniform and their beaded nature is almost lost. The nucleolus stains uniformly and appears to be homogeneous. It seems to remain free without any apparent connection with the spireme, though occasionally it lies within the tangle of the spireme. At this stage the volume of the nucleus as well of the nucleolus appears to have increased. The pollen mother cells which were polygonal in outline and packed closely inside the anther loculus now begin to round off, and become separated from one another.

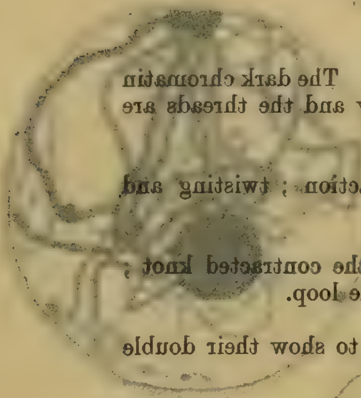
The dark-staining chromatin bodies which were found to be distributed all over the leptotene threads in the early stages, can no longer be detected on the spireme but a few dark bodies are found to lie on it. These bodies are much bigger than those noted before and are fewer in number. Occasionally it is noted that the threads are very closely connected with these bodies. Plate XLI, fig. 11 shows the presence of such a dark body which lies at the periphery of the nucleus and is in close contact with the spireme. The presence of these dark bodies and the disappearance of the smaller ones observed in the early stages, may be accounted for by the fact that during synizesis the smaller bodies have coalesced to form the bigger bodies now observed.

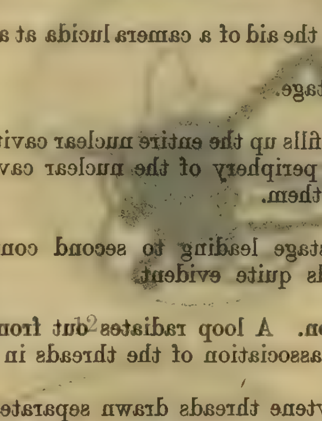
It is rather difficult to make out the dual nature of the fully opened spireme This might be due to the close union of the homologues at the synaptic stage. This observation seems to agree with that of Beal's [1932] interpretation of the open spireme stage of *Nothoscordum*. He suggests that sometimes the spireme threads appear to be single in the main, but careful examination shows that each is composed of two threads so closely twisted and mixed as practically to hide its double nature. In this material also careful examination reveals that the spireme threads though apparently single are in reality double. The double nature of the thread becomes more apparent as the spireme unravels itself.

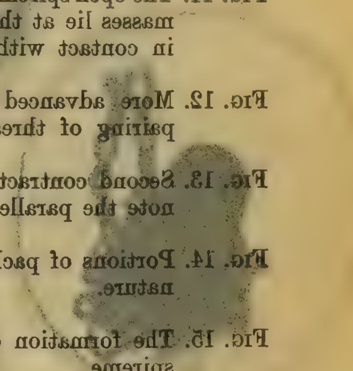
Second contraction.—Although the spireme appears to fill the nuclear cavity yet it does not completely unravel itself. The presence of a loose knot is seen in many preparations. At this stage the threads begin to condense and become comparatively thick. The spireme is pulled away centrally from the nuclear membrane and gradually forms a dark mass. This stage corresponds to the second contraction stage (Plate XLI, figs. 12 and 13). The contracted mass lies either towards the periphery, or at the centre of the nuclear cavity. The nucleolus is sometimes observed to lie within the tangled mass of threads which appears as a dark mass. Loops of various forms are seen to radiate from the contracted mass. It may be mentioned here that the dark bodies which were noted on the spireme before are no longer visible now,



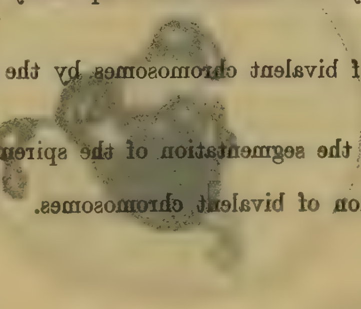


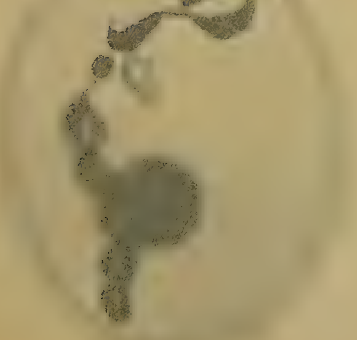



2



11





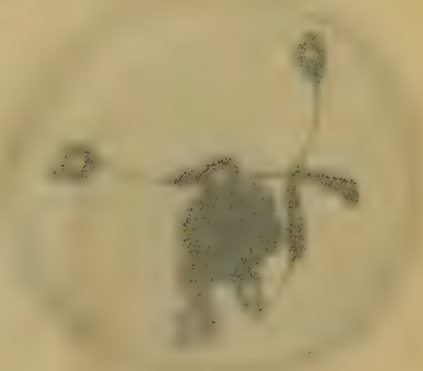


PLATE XLI

(All figures were drawn with the aid of a camera lucida at a magnification of × 3,000)

FIG. 10. Hollow spireme stage.

FIG. 11. The open spireme fills up the entire nuclear cavity. The dark chromatin masses lie at the periphery of the nuclear cavity and the threads are in contact with them.

FIG. 12. More advanced stage leading to second contraction ; twisting and pairing of threads quite evident.

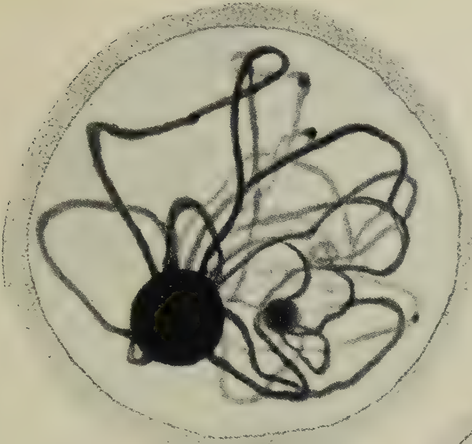
FIG. 13. Second contraction. A loop radiates out from the contracted knot ; note the parallel association of the threads in the loop.

FIG. 14. Portions of pachytene threads drawn separately to show their double nature.

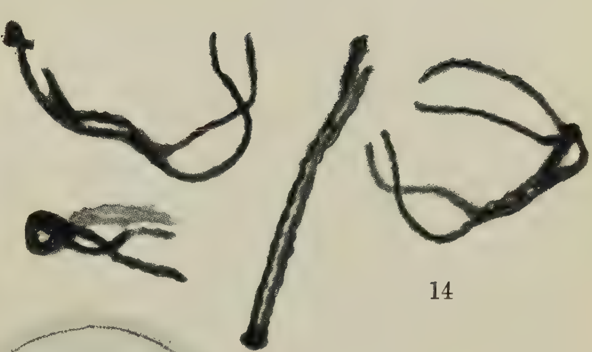
FIG. 15. The formation of bivalent chromosomes by the segmentation of the spireme.

FIG. 16. A later stage in the segmentation of the spireme.

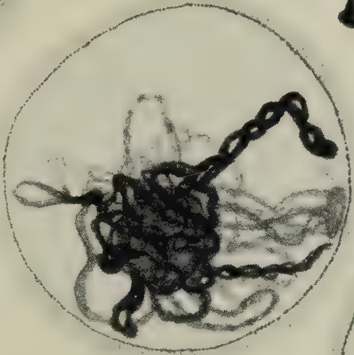
FIG. 17. Mode of formation of bivalent chromosomes.



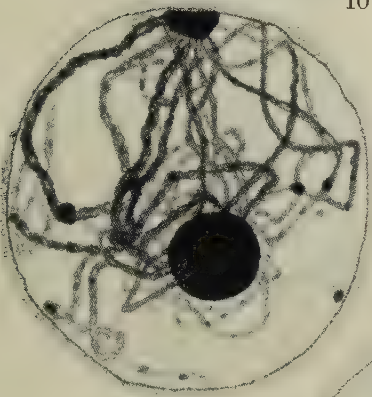
10



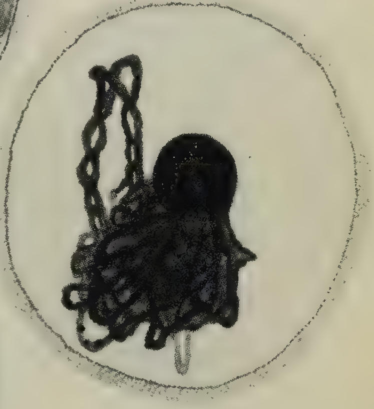
14



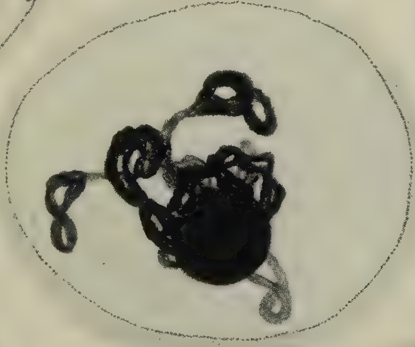
12



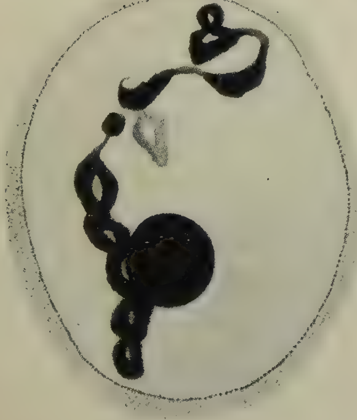
11



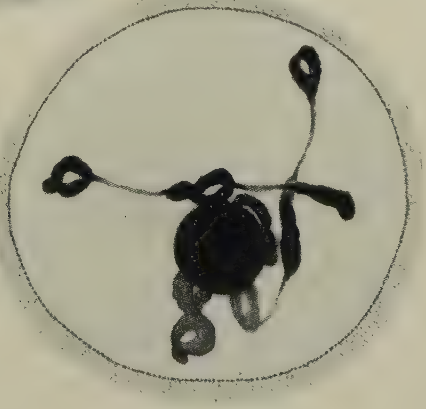
13



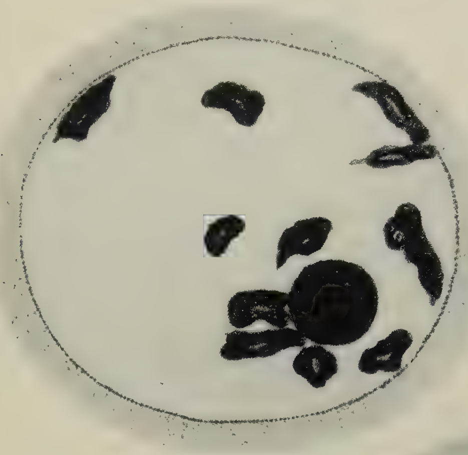
15

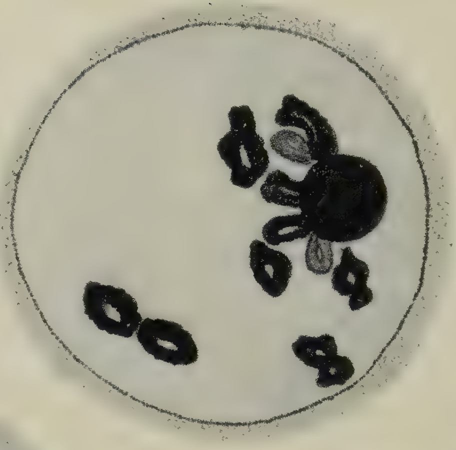


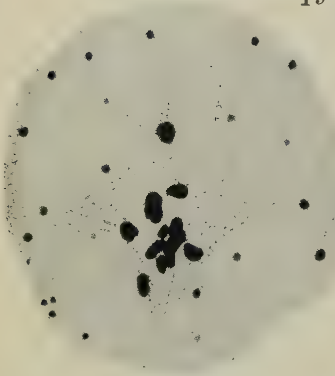
16

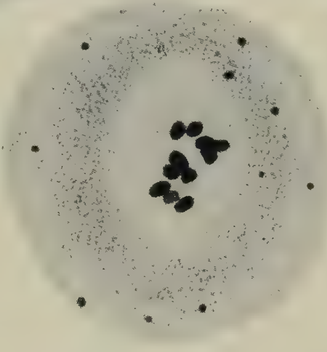


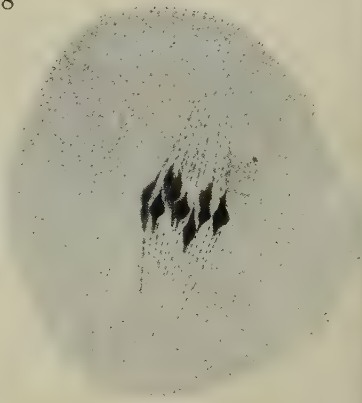
17

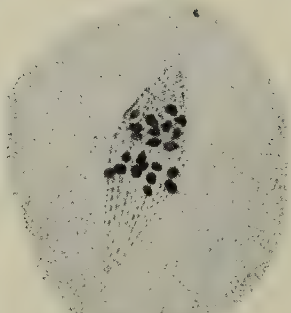

19

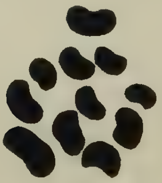

18

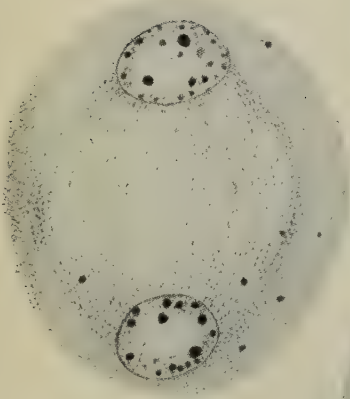

21

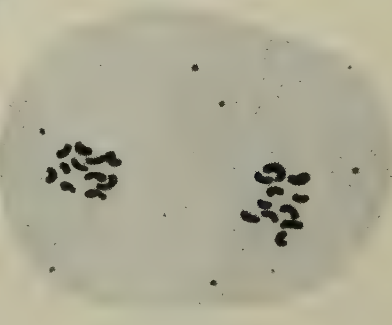

20


23


24


22


25


27

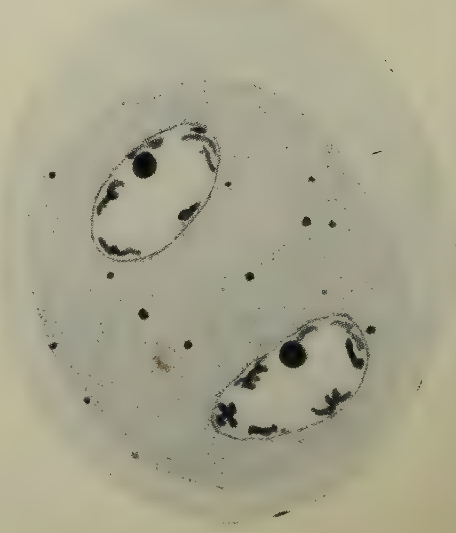

26

PLATE XLII

(All figures were drawn with the aid of a camera lucida at a magnification of ×3,000, except figure 22 which has been drawn at a magnification of ×4,200).

FIG. 18. Early diakinesis showing the variability in size of the eleven bivalent chromosomes. The bivalent pairs are not morphologically identical and no heteromorphic pair is present.

FIG. 19. Diakinesis.

FIG. 20. Origin of spindle fibres ; a weft of fibres is seen in the nuclear cavity just after the dissolution of the nucleolus and the nuclear membrane. Note also the preponderance of dark bodies in the cytoplasm.

FIG. 21. Origin of multipolar spindle. Note also the dark cytoplasmic bodies.

FIG. 22. Equatorial plate. Heterotypic division.

FIG. 23. Early heterotypic anaphase showing mode of attachment of the chromosomes to the spindle fibres.

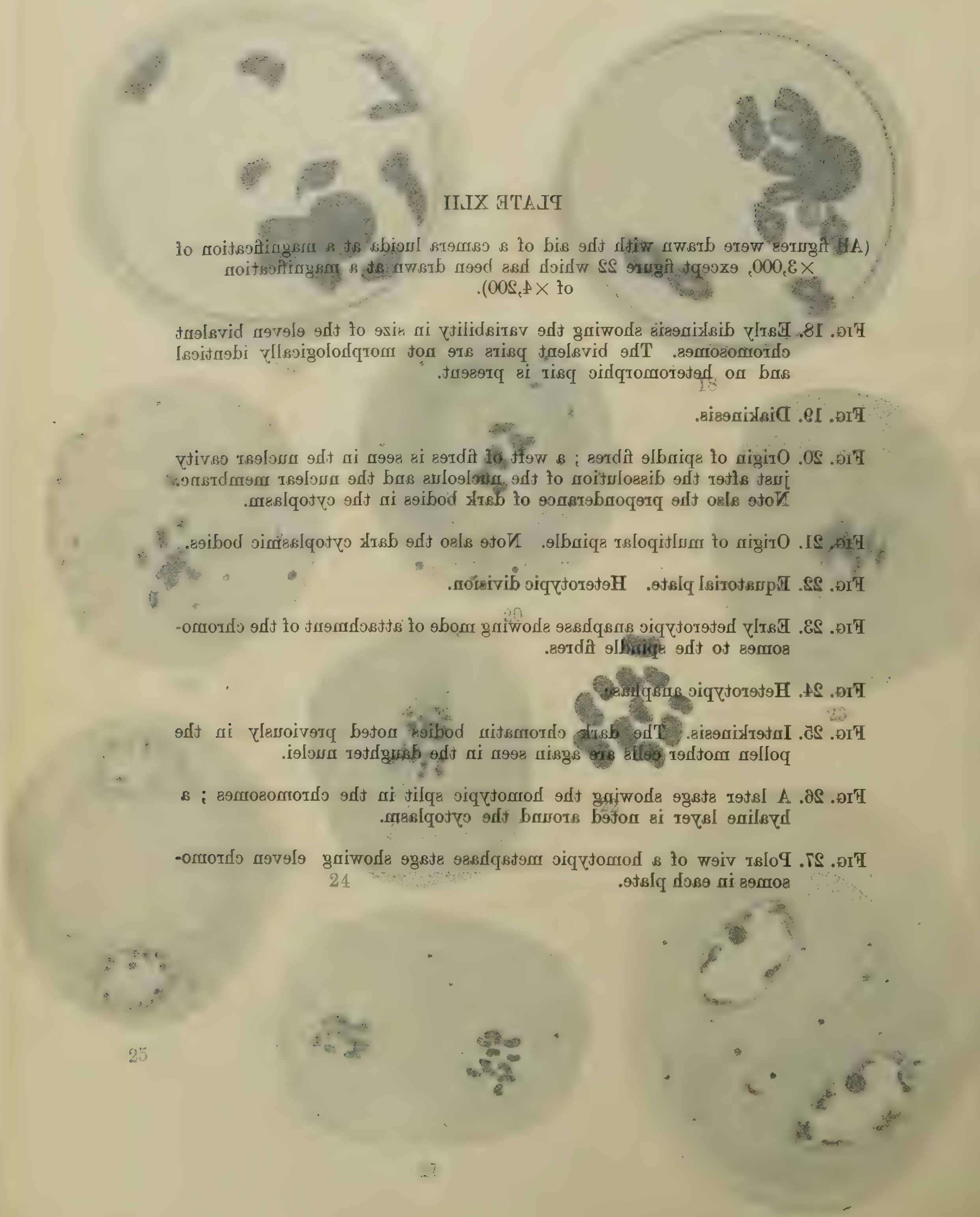
FIG. 24. Heterotypic anaphase.

FIG. 25. Interkinesis. The dark chromatin bodies noted previously in the pollen mother cells are again seen in the daughter nuclei.

FIG. 26. A later stage showing the homotypic split in the chromosomes ; a hyaline layer is noted around the cytoplasm.

FIG. 27. Polar view of a homotypic metaphase stage showing eleven chromosomes in each plate.

PLATE XLII



During the process of second contraction pairing and twisting of the threads become increasingly evident. Plate XLI, fig. 13 represents a stage where one loop is seen to radiate from the contracted mass and is composed of two threads which have fused at places. Such pairing and twisting appear to be of general occurrence and can be seen in almost every preparation showing this stage. Sometimes the pairing homologues meet one another at certain points and fuse and appear as a single thread.

Though various forms of loops have been noted at this stage yet careful observation of these loops does not lend any support to the telosynaptic mode of chromosome conjugation in this plant. In no case was definite arrangement of the loops noted, nor do the loops suggest that they are composed of two univalents arranged end to end. It has been noted by some investigators that in telosyndesis the number of loops in the second contraction stage correspond to the haploid chromosome number of the plant. No evidence in that direction was obtained in the course of the present investigation. The number of loops observed was very small, and did not at all correspond to the haploid chromosome number of the plant ; closer observation showed that the loops were composed of two univalents arranged side by side (Plate XLI, fig. 14). Further evidence in support of this statement is obtained during the segmentation of the spireme to form the bivalent chromosomes (Plate XLI, figs. 15, 16 and 17).

Diakinesis.—The contracted mass unravels a little and shortly afterwards segmentation of the spireme begins (Plate XLI, fig. 15). Careful examination at this stage shows the mode of arrangement of the homologous chromosomes leading to the formation of the gemini. It is now seen that the two homologues have associated side by side. As the chromosomes shorten and thicken it appears that the chromatin elements of the segmented spireme condense towards the centre, leaving behind the pale non-staining linin thread. These linin threads are in some cases seen to be connected to groups of bivalent chromosomes. Gradually the chromosomes condense still further and the linin connections become obscure. Plate XLI, figs. 15, 16, 17 illustrate the method of formation of the bivalent chromosomes. At early diakinesis eleven bivalent chromosomes are seen to be scattered in the nuclear cavity (Plate XLII, fig. 18). The lateral association of the threads observed during the early stages of meiosis and the process of formation of bivalent chromosomes clearly indicate a parasynaptic method of chromosome conjugation in this plant.

The bivalent chromosomes gradually condense still further and become compact in form. They show some variation as regards size. Plate XLII, fig. 19 represents a typical diakinetic stage. In this figure one large pair, several intermediate pairs, and one small pair of chromosomes may be noticed. Critical examination at this stage failed to reveal any cytological evidence regarding the dioecious nature of the plant. The configurations of the chromosomes are the strongest evidence on the point.

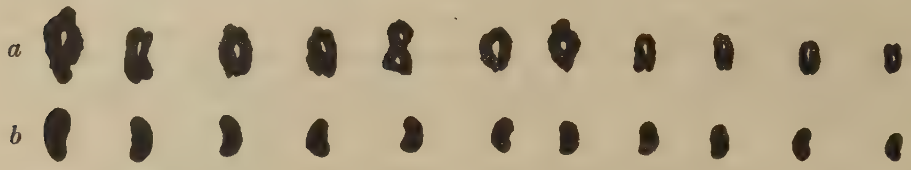


FIG. 1. Chromosome configuration in diakinesis (*a*) and heterotypic metaphase (*b*) (×3,500)

Spindle formation.—Soon after the disappearance of the nucleolus and the nuclear membrane some felt-like material of achromatic nature is seen inside the nuclear cavity (Plate XLII, fig. 20). These seem to radiate towards the periphery. Gradually these fibre-like processes become arranged and give rise to the multipolar spindle (Plate XLII, fig. 21). The chromosomes at first lie scattered on the multipolar spindle but gradually they become grouped. The first indications of the transformation of the multipolar spindle to the bipolar condition is noted at the extremities of the multipolar spindle. The peripheral fibres re-organise themselves and gradually a bipolar condition is reached. With the formation of the bipolar spindle the chromosomes align themselves on the equatorial region.

In some pollen mother cells a slightly thickened cytoplasmic area was noted around the nuclear cavity (Plate XLII, fig. 20). This zone might be an artifact due to the action of fixing fluids. Many investigators refer to this as the perinuclear zone and believe that the spindle originates from this zone. Denham [1924] found it to be the case in *Gossypium barbadense* and believes that the spindle is extra-nuclear in origin. Evidences obtained in the course of this investigation, however, show that the spindle is intra-nuclear in origin as described by Kirkwood [1907] in *Micrampelis lobata.*

Heterotypic division.—The heterotypic spindle is typically bipolar and the chromosomes which have by this time undergone the maximum amount of condensation and have become more or less bean-shaped, are arranged on the equatorial region of the spindle at metaphase. The bivalent nature of the chromosomes is difficult to make out at this stage. The spindle is comparatively small and the cytoplasm appears to be homogeneous throughout. Polar views of equatorial plates at metaphase show eleven distinct bivalent chromosomes (Plate XLII, fig. 22). The number corresponds to that observed during diakinesis. At this stage size variations of the chromosomes are very apparent. According to their form they may be roughly classified as follows :—one large, five median, four sub-median, and one small. They could be represented by the following formula :—

$$1L + 5M + 4SM + 1S.$$

The arrangement of the chromosomes with respect to one another is also variable. Very often the largest and the smallest chromosome seem to lie at the periphery of the spindle, but this position does not appear to be fixed.

During early anaphase each member of a bivalent pair separates a little and on account of the telomitic attachment of the traction fibres they show attenuated ends. Thus each member of a bivalent pair assumes the form of a ' V ' with the angular ends pointing towards the poles of the spindle (Plate XLII, fig. 23). As the univalents migrate towards the poles they become round in form (Plate XLII, fig. 24). On reaching the poles the chromosomes aggregate together, but they retain their identity for some time. The spindle fibres at this stage become stretched from pole to pole. A hyaline zone is noted around the chromosomes and this area looks lighter than the surrounding cytoplasm. Gradually two daughter nuclei are organised (Plate XLII, fig. 25) each being bounded by a delicate membrane and containing usually one, but occasionally two or more nucleoli. The identity of the chromosomes is altogether lost and a granular substance and dark chromatin granules are found in the inter-kinetic nuclei. It will be interesting to note in this connection that similar dark chromatin granules were noted during the resting condition of the pollen mother cells. The daughter nuclei formed are oval or round in form. Very often striations of the cytoplasm are noted between the nuclei, but these are gradually absorbed. Castetter [1926] has observed the presence of a ' cell plate ' both in the heterotypic and homeotypic spindles in *Cucurbita maxima* and stated that the cell plates soon disorganise without performing any definite function. Passmore [1930] working on the same material has failed to observe the presence of such a cell plate after any division. In this material also no trace of any cell plate has been noted.

Homeotypic division.—The first evidence of homeotypic division is noted in the reticulum of the inter-kinetic nuclei. The chromosomes are gradually organised from the faintly staining reticulum and the homeotypic split becomes apparent in the chromosomes at this stage. Occasionally the two split halves lie at right angles to one another (Plate XLII, fig. 26). Very soon the nucleus and the nuclear membrane of the inter-kinetic nuclei disorganise and multipolar spindles are noted which later become bipolar. The chromosomes then align themselves on the equatorial region of the spindle. The spindles have been observed to lie either parallel to or at right angles to one another. Plate XLII, fig. 27 shows polar views of two homeotypic metaphase spindles. Eleven chromosomes are seen in each case and they show the same variation in size as has been noted in the bivalent chromosomes.

The chromosomes on reaching the poles soon coalesce and lose their identity. Very soon a nuclear membrane is organised and nucleoli make their appearance. Generally one nucleolus may be noted in each nucleus but occasionally more than one has been noted. The cytoplasm around the nuclei seem to be homogeneous except for the dark cytoplasmic bodies.

Cytokinesis.—The nuclei formed as a result of the homeotypic division are found to lie in the cytoplasm surrounded by a hyaline layer. Constrictions appear at the periphery of the cytoplasm and progress inwards between the nuclei (Plate XLIII, fig. 29). Along with this the hyaline layer which surrounds the protoplast flows inwards along the grooves of the cytoplasm. The cytoplasmic furrows and the hyaline projections become almost mixed up and it is difficult to discriminate them. The furrows cut inwards progressively and meet in the centre of the cell separating the four young pollen grains, which are at this stage surrounded by the hyaline layer alone. The cytoplasm of the young pollen grains very soon secretes walls of their own. The pollen grains are somewhat oval at this stage.

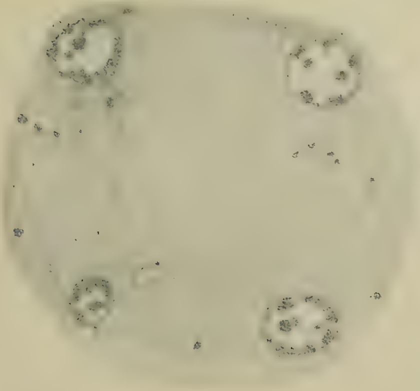
Quadripartition of the microspores by furrowing appears to be common amongst the angiosperms. Castettar [1926], Hemlich [1927], Passmore [1930] and Sutaria and Asana [1932] have all observed it in the various Cucurbitaceous plants they have studied.

CYTOPLASMIC BODIES

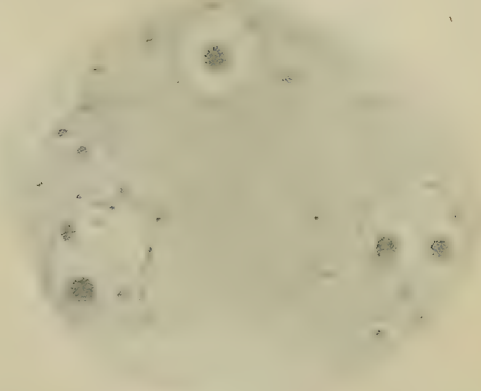
It has already been mentioned that certain cytoplasmic bodies were constantly noted in the pollen mother cells during the heterotypic and homeotypic divisions. In the resting condition of the pollen mother cells, the cytoplasm appears to be quite homogeneous and uniform throughout and no trace of any foreign element is noted. At the prophase stage of the nucleus these cytoplasmic bodies make their appearance. They are not very conspicuous at this stage, nor do they stain intensely. During late prophase an increase in their number is noted and during synizesis the cytoplasm throughout is seen to be spotted with these dark bodies. These bodies are not all of equal size, some are large others are small. Plate XLII, fig. 21 shows a multipolar spindle stage where cytoplasmic bodies of various sizes can be seen scattered in the cytoplasm. The cytoplasmic bodies can be constantly seen throughout the successive stages of both the divisions. Prior to cytokinesis these cytoplasmic bodies appear to shift a little and arrange themselves more or less around the nuclei. Plate XLIII, fig. 29 shows such a stage where it can be seen that the cytoplasmic bodies have collected close to the cytoplasm of the neighbouring nuclei, so that they may be distributed in the cytoplasm of the young pollen grains ; where they eventually get distributed (Plate XLIII, fig. 30). Thus it seems that these bodies appear quite early in the history of the young pollen mother cells, and through successive stages of both the divisions they are passed on to the cytoplasm of the pollen grains.

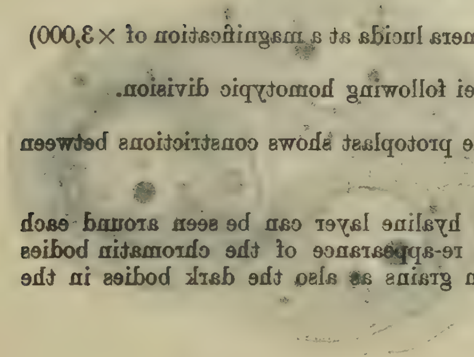
CHROMOSOME NUMBER

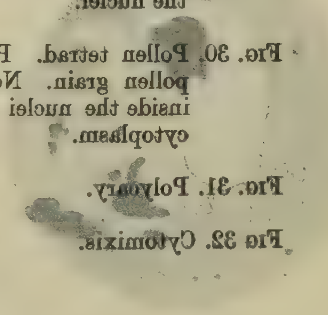
In properly fixed and stained material, mitotic divisions of the root-tip cells of both the male and the female plants show the chromosomes very well. Repeated counts of perfect plates show that the diploid chromosome number of *Trichosanthes dioica* is 22 (Figs. 4 and 5). It will be seen very clearly from the figures



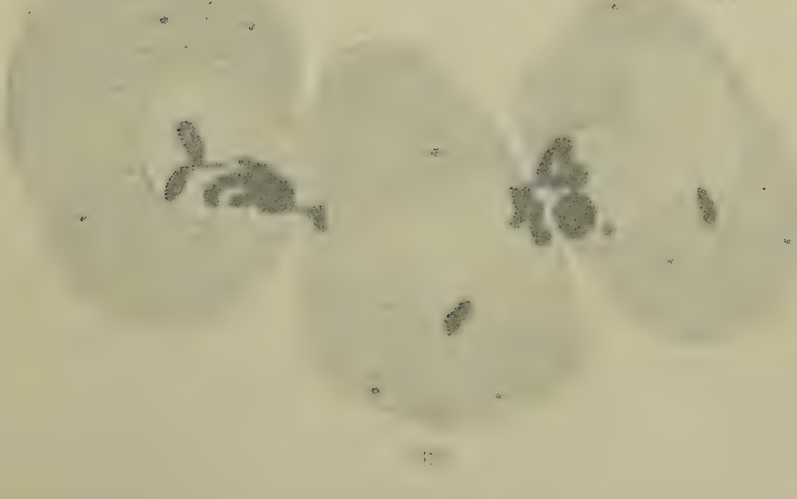
28







31



32

PLATE XLIII

(All figures drawn with the aid of a camera lucida at a magnification of × 3,000)

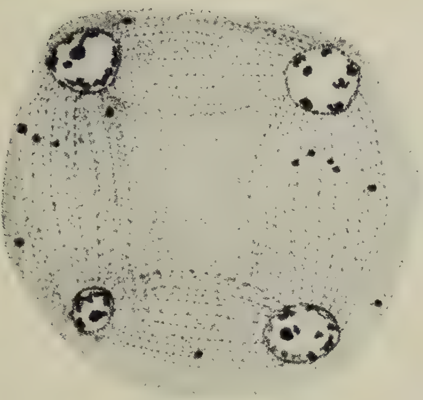
Fig. 28. Bilateral arrangement of nuclei following homotypic division.

Fig. 29. Beginning of cytokinesis. The protoplast shows constrictions between the nuclei.

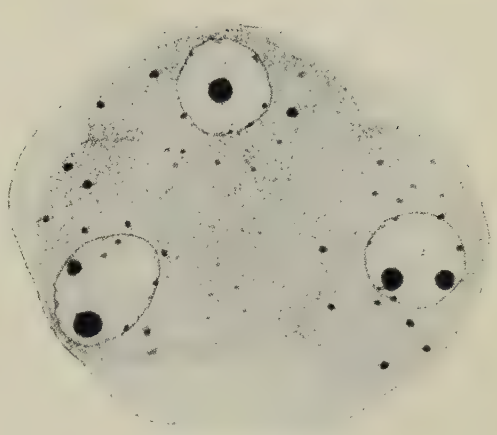
Fig. 30. Pollen tetrad. Presence of a hyaline layer can be seen around each pollen grain. Note also the re-appearance of the chromatin bodies inside the nuclei of the pollen grains as also the dark bodies in the cytoplasm.

Fig. 31. Polycary.

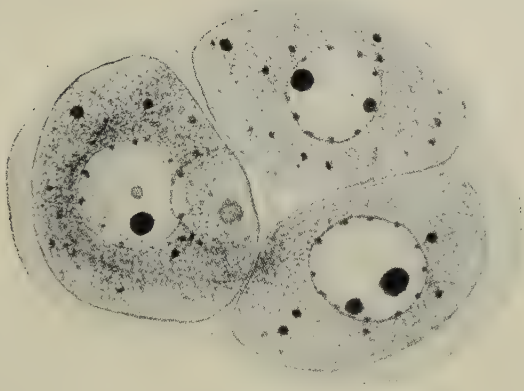
Fig 32. Cytomixis.



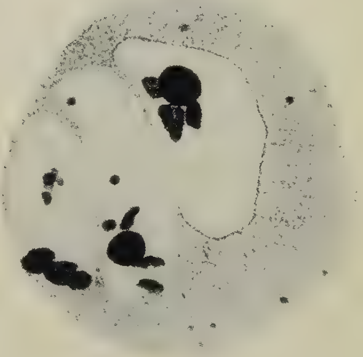
28



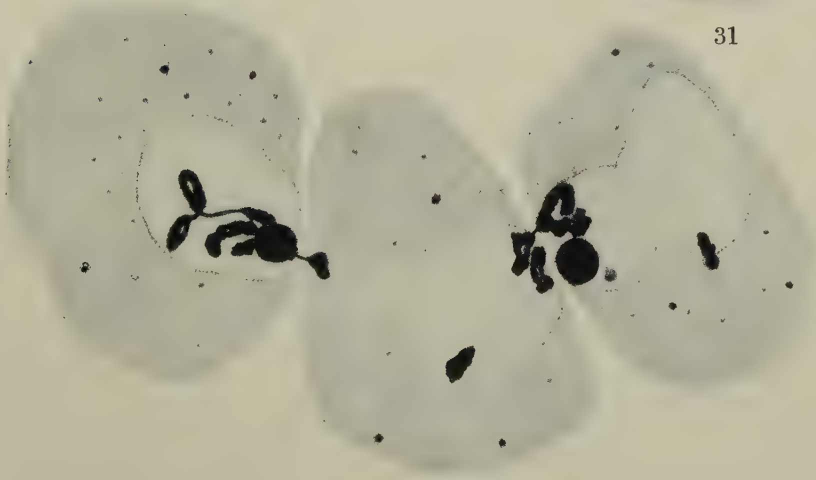
29



30

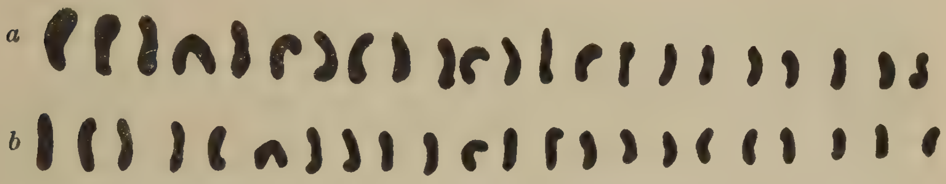


31

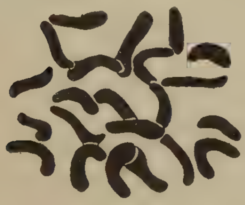


32

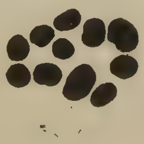
that the chromosomes differ in size, some being large, others intermediate and small in size. On comparing the size of the chromosomes of the male and the female plants (Fig. 2, *a* and *b*) it appears that the chromosomes of the female plant are slightly bigger in size than those of the male. The haploid number of chromosomes as determined for the male plant is eleven. The haploid number of chromosomes of *Trichosanthes anguina* is also determined in order to note the chromosome number of the plant, which is monoecious. It is found that the plant possesses eleven haploid chromosomes (Fig. 3).



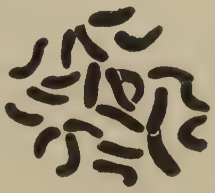
2



4



3



5

FIG. 2. Somatic chromosomes of the female (*a*) and male plants (*b*) arranged serially to show the size and form of the chromosomes (×3,500). FIG. 3. Heterotypic metaphase plate of *Trichosanthes anguina* showing eleven bivalent chromosomes (×3,500). FIGS. 4 and 5. Somatic chromosomes of the female and male plant respectively (×3,500).

ABNORMALITIES

Various forms of abnormalities have been noted during the meiotic division. During synizesis and later stages various forms of degenerating processes have been noted in the pollen mother cells. During synapsis it has often been noted that the spireme before it reaches the maximum contraction breaks up into fragments and gradually the whole nucleus and even the pollen mother cells shrivel up and appear as a black mass. Degeneration of the pollen mother cells are also noted in the open spireme stage. In such cases the spireme fragments before completely unravelling, and ultimately the entire pollen mother cell degenerates. Degeneration processes become more marked especially during second contraction stage and diakinesis. In the second contraction stage it is often noted that the contracted spireme with the nucleolus fragments into bits and ultimately the whole nucleus degenerates and blackens. Polycary and cytomixis are very commonly noted in the diakinetic stage. Sometimes the chromatin matter extrudes out from the nucleus and tries to organise separate nuclei in the cytoplasm of the pollen mother cells. In these diminutive nuclei no nuclear membrane can be demonstrated, but a lining of cytoplasm marks the outline of these nuclei. Generally these diminutive nuclei are much smaller than the mother nucleus, and are ultimately found to disorganise.

DISCUSSION

Chromatin bodies in the nuclei of the microspore mother cells.—Dark-staining chromatin bodies have been observed in the resting condition of the nucleus during meiotic mitosis. They were mostly adpressed to the nuclear membrane. Apparently similar bodies have been observed by many investigators in a number of plants. De-Litardiere [1921] has observed these bodies in the early stages of the meiotic divisions of *Azolla*. He described them as pro-chromosomes and stated that they orient themselves on the metaphasic plate without taking any part in the formation of the spireme. Janaki Ammal [1932] noted paired arrangement of these bodies in *Nicandra physaloides*. She believes that these bodies represent late stages in the expansion of the chromosomes of the resting nucleus prior to the formation of the reticulum. In the microsporocytes of *Equisetum arvense* Beer [1913] has noted paired chromatic bodies and he considers their presence as a chance phenomenon depending on 'natural or artificial tensions'. In *Trichosanthes dioica*, the chromatin bodies were never observed to lie in pairs and their number did not correspond to the number of chromosomes in either divisions. They were found to be constantly present in the reticulum up to the second contraction stage, after which and along with the formation of the chromosomes they altogether disappeared. These chromatin bodies were again seen in the daughter nuclei after the disappearance of the chromosomes. During the homeotypic division also they disappeared along with the formation of the chromosomes and re-appeared in the pollen nuclei. The disappearance of these dark chromatin bodies during the formation of the chromosomes and their re-appearance in the re-constructed nuclei have led the writers to believe that these bodies pass from cell to cell through the chromosomes. Their behaviour appears to be different to that of similar chromatin bodies observed by other investigators and referred to as pro-chromosomes.

Chromosome conjugation.—While it is generally accepted that meiosis consists in the segregation of homologous chromosomes there exists quite divergent views among cytologists regarding the method of conjugation of homologous chromosomes. At present there are two principal theories—the Parasynaptic and the Telosynaptic theories—with a number of eminent cytologists as upholders of each theory. The Parasynaptic theory was first propounded by Von Winiwater in 1901 and later on supported by the Louvain school, Gregoire and others as pioneers. The Telosynaptic theory on the other hand was first proposed by Montogomery in 1903 and later on by Farmer and Moore [1905]. During the last thirty years quite an amount of cytological data have accumulated and both the theories have been supported by a number of eminent cytologists. In many species, however, meiosis has been interpreted differently by different workers. Recently Latter [1932] has shown that the side-by-side association of the threads in *Lathyrus odoratus* is due to fixation and confirmed her original observation, *viz.*, telosynaptic mode of chromosome conjugation in the plant. According to Maeda [1930] however, the mode of chromosome conjugation in *Lathyrus odoratus* is of the parasynaptic type.

Careful examination of the leptotene threads in *Trichosanthes dioica* reveals the fact that at places it is double. The lateral approximation of the threads can be made out in most of the preparations. During the process of contraction of the spireme, duality of the threads can also be made out in most preparations showing this stage. Passmore [1930] working on a number of Cucurbitaceous plants has stated that " the leptotene thread show some tendency to pair but it is impossible to tell whether the apparent pairing which occurs here and there is significant or accidental ". Kirkwood [1907] has also found longitudinal cleavage of the thread in the pre-synizetic stage of *Micrampelis lobata.* Asana and Sutaria [1932] who worked on *Luffa aegyptiaca* observed a ' split ' in the open spireme stage (*vide* Asana and Sutaria's Fig. 4 in Plate I), and from this alone they conclude " that the method of chromosome pairing fall in line with that of typical parasynapsis ". In the material studied, the threads appear to be uniform in the open spireme stage and this is probably due to the very close approximation of the homologues during synizesis.

The close association of the homologues noted in the process of second contraction and the method of formation of the bivalent chromosomes unquestionably confirms the parasynaptic mode of chromosome conjugation in this plant. The close association of the threads noted in almost every preparation showing the second contraction stage gives ample evidence to show that the chromosomes have conjugated side by side and the split is not due to the telophasic alveolisation of the chromosomes. The segmentation of the spireme adds further evidence to this contention. The bivalent chromosomes are formed by transverse segmentation of the spireme as represented in Plate XLI, figs. 15, 16 and 17.

The adherents of the telosynaptic theory claim that chromosome conjugation takes place in the second contraction stage, but in this material second contraction is not significant from the point of chromosome conjugation as observed from the mode of formation of the bivalent chromosomes. The spireme already double, segments transversely, and in almost every preparation showing this stage the double threads as one would expect from such a spireme is clearly seen. The closed loops that are occasionally seen are due to the condensation of the fragmented spireme and not due to folding.

Sex chromosome.—The presence of sex chromosomes has been demonstrated in a very few angiosperms. Lindsay [1930] in her paper on ' the chromosomes of some dioecious angiosperms ' has given a complete account of the dioecious angiosperms which have been studied cytologically from the stand-point of sex determination.

In the family Cucurbitaceae the presence of a sex chromosome of the *X Y* type has been reported by Sionoto [1928] in *Trichosanthes japonica*. This appears to be the only reported case of the presence of a sex chromosome in this family. One of the considerations which led the writers to undertake the present investigation was to note if the presence of a sex chromosome is responsible for the dioecious nature of *Trichosanthes dioica*. Critical examination of the various stages particularly diakinesis and heterotypic anaphase have failed to reveal the presence of hetero-chromosomes. The bivalent pairs observed in diakinesis do not indicate the presence of a heteromorphic pair as has been observed by Santos [1923, 1924] in *Elodea canadensis* and *Elodea gigantea*. The bivalent pairs noted at diakinesis are serially represented in Fig. 1. It will be seen that the homologue of each pair is morphologically identical. The anaphasic movement of the chromosomes show that the homologues separate regularly, and the separated univalents of a bivalent pair are also morphologically identical.

Chromosome numbers were determined from the root-tip cells of both male and female plants in order to determine if either of the plants possessed an extra chromosome, as has been reported for *Dioscorea sinuata* (*X O* type) [Meurman, 1925]. Critical examination of a large number of chromosome plates showed that the chromosome complements of both the plants were the same (*viz.* $2n$-22). When serially arranged as has been represented in Fig. 2, *a* and *b*, it appears that the chromosomes of the female plant are slightly bigger than those of the male. Beyond this no other apparent difference has been noted.

Lindsay's [1930] investigation on *Bryonia dioica*, a dioecious Cucurbitaceous plant, showed that no hetero-chromosome was present. The present investigation on *Trichosanthes dioica* also failed to show the presence of a sex chromosome which could be correlated with the dioecious nature of the plant.

Cytoplasmic bodies.—Kirkwood [1907] has observed some cytoplasmic bodies in the pollen mother cells of Cyclanthera. He described them as extra-nuclear nucleoli and assigned their origin to some " short crooked darkly staining fibres which are parallel to one another, which are found on one side of the nucleus and about half way from the nucleus to the wall ". As soon as these fibres disappear " certain minute darkly staining granules may be seen scattered prominently throughout the cytoplasm ". No such cytoplasmic fibres have been noted in the course of the present investigation. In *Trichosanthes dioica* presence of these cytoplasmic bodies have been observed from the early stages of the pollen mother cells. They are present in the cytoplasm throughout the divisional stages of the pollen mother cells and are also noted in the cytoplasm of the young microspores.

SUMMARY

1. In the resting nucleus of the microspore mother cells, some dark-staining chromatin granules closely adpressed to the nuclear membrane have been noticed. These bodies were later observed to lie scattered on the spireme of the pollen mother cells and gradually disappeared with the formation of the chromosomes. They were noted again in the inter-kinetic nuclei after the dissolution of the chromosomes. With the reorganisation of the chromosomes in the homotypic division they were again lost sight of and were later found in the nuclei of the pollen grains. These dark chromatin bodies appear to pass from cell to cell through the chromosomes.

2. Parallelism of the leptotene threads has been noted at places during the prophase. Prior to synizesis the parallelism of the threads become increasingly evident. In the open spireme stage, however, it was difficult to make out the dual nature of the spireme. During second contraction, clear evidence as to the side-by-side pairing of the homologous chromosome leading to the formation of gemini was obtained. The spireme segments transversely. The chromatin matter of the segmented spireme condense towards the centre leaving at both ends faintly staining linin elements, which are gradually absorbed and the chromosomes assume the typical bivalent form.

3. In diakinesis eleven bivalent chromosomes were observed.

4. No evidence as to the presence of a sex-chromosome was obtained during the meiotic division.

5. Cytokinesis takes place by the process of furrowing.

6. The chromosome numbers of the male and the female plants as determined from the somatic cells of the root tips was found to be 22 in each case. The chromosomes of the female plant were found to be slightly bigger than those of the male.

7. *Trichosanthes anguina* was found to contain eleven haploid chromosomes.

8. Various forms of abnormalities have been observed during the development of the microspores. During diakinesis polycary and cytomixis are the most common irregularities noted. Irregular movement of the chromosomes has been noted during the meiotic division of the pollen mother cells.

9. Certain dark-staining bodies have been observed in the cytoplasm in the pollen mother cells during meiosis. These bodies orient themselves around the grand daughter nuclei and are distributed to the cytoplasm of the young pollen grains by cytokinesis.

REFERENCES

Asana, J. J. and Sutaria, R. N. (1932). *Ind. Jour. Bot. Soc.* **11**, 181-187.

Beal, J. M. (1932). *Bot. Gaz.* **93**, 278-295.

Beer, R. (1913). *Ann. Bot.* **27**, 643-659.

Boenicke, V. (1911). *Ber. Deut. Bot. Ges.* **29**, 59-65.

Castetter, F. F. (1926). *Amer. Jour. Bot.* **13**, 1-10.

Denham, H. J. (1924). *Ann. Bot.* **38**, 407-432.

Farmer, J. B. and Moore, J. E. S. (1905). *Quart. Jour. Micros. Sci.* **28**, 489-557.

Gates, R. R. and Rees, E. M. (1921). *Ann. Bot.* **35**, 364-398.

Heimlich, L. F. (1927). *Proc. Nat. Acad. Sci.* **13**, 364-398.

Janaki Ammal, E. K. (1932). *La Cellule* **41**, 89-110.

Kirkwood, J. E. (1907). *Bull. Torr. Bot. Club.* **34**, 221-242.

Kozhuchow, S. (1925). *Bull. Appl. Bot. Pt. Breed.* **14**, 89-96.

Latter, J. (1932). *Ann. Bot.* **46**, 807-811.

Lindsay, R. H. (1930). *Amer. Jour. Bot.* **17**, 152-174.

De-Litardiere, R. (1921). *La Cellule* **31**, 225-473.

Lundegardh, H. (1912). *Beitr. Biol. Pflanzen* **2**, 373-542.

——————— (1914). *Arch. Zellforsch.* **13**, 145-157.

Maeda, T. (1930). *Memo. Coll. Sci. Kyoto. B.* **5**, 89-123.

Meurman, O. (1925). *Soc. Sci. Fenn. Comm. Biol.* **2**, No. 3.

Passmore, S. F. (1930). *Bot. Gaz.* **90**, 213-223.

Santos, J. K. (1923). *Bot. Gaz.* **75**, 42-59.

———————(1924). *Bot. Gaz.* **77**, 353-376.

———————(1928). *Phill. Jour. Sci.* **37**, 417-437.

Sinoto, Y. (1928). *Proc. Imp. Acad. Sci. (Tokyo)* **4**, 175-177.

Strasburger, E. (1910). *Jahrb. Wiss. Bot.* **48**, 427-520.

Whitaker, W. T. (1933). *Bot. Gaz.* **94**, 780-790.

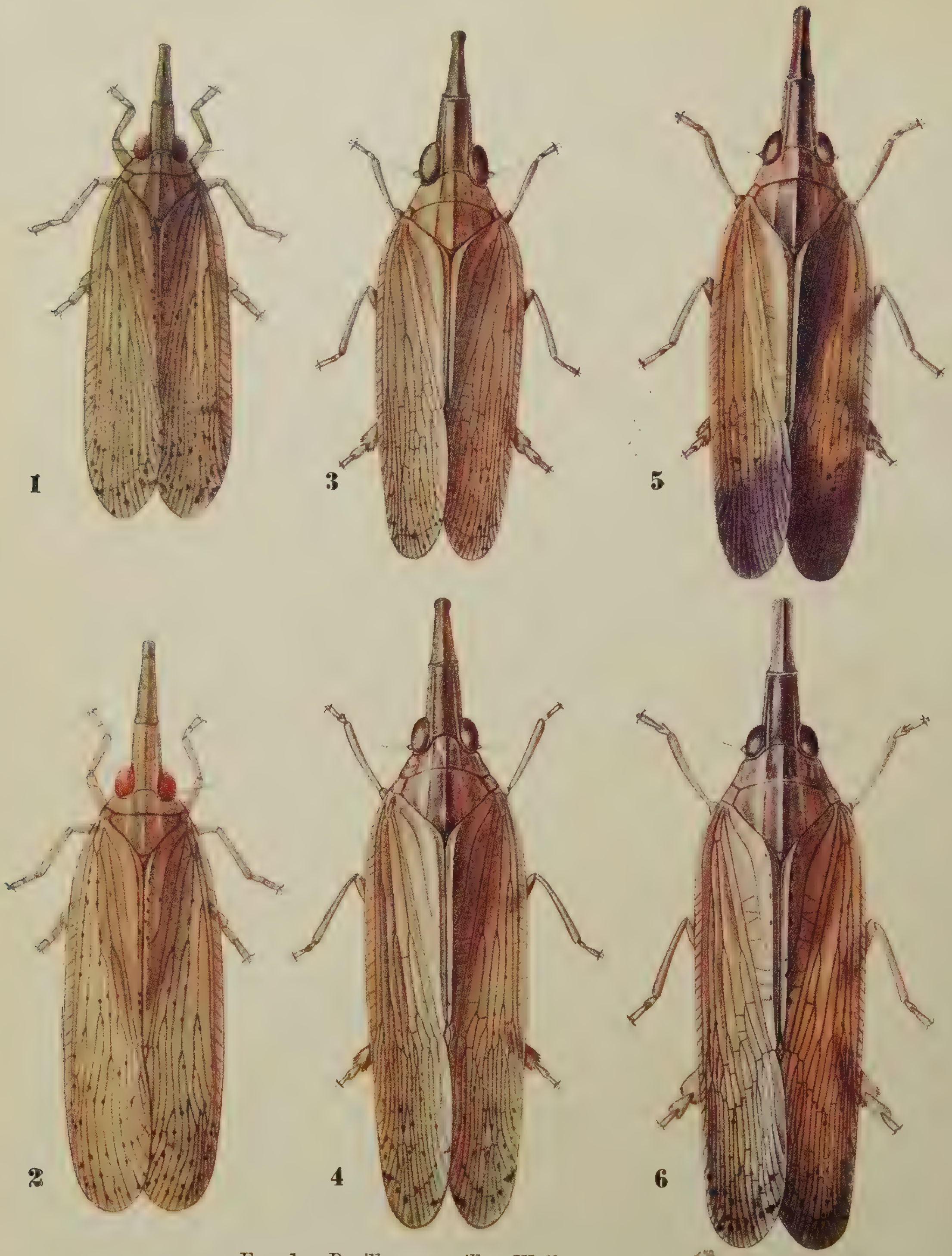


FIG. 1. *Pyrilla perpusilla* Walk., male (× 7)
FIG. 2. ,, ,, female (× 7)
FIG. 3. *Pyrilla aberrans* Dist. (nec. Kirby), male (× 7)
FIG. 4. ,, ,, ,, female (× 7)
FIG. 5. *Pyrilla pusana* Dist., male (× 7)
FIG. 6. ,, ,, ,, female (× 7)

ON THE *PYRILLA* PEST OF SUGARCANE IN INDIA

BY

HEM SINGH PRUTHI, M.Sc., Ph.D. (Cantab.), F.N.I.

Imperial Entomologist, New Delhi

(Received for publication on 25th February 1937)

(With Plates XLIV-XLVI)

In the literature on the pests of sugarcane in India three species of *Pyrilla* are recorded to be of common occurrence, *viz.*, *P. perpusilla* Walk., *P. aberrans* Kirby, and *P. pusana* Dist. The distinguishing features of these species are given in the *Fauna of British India*, *Rhynchota*, Vols. III and VII by Distant. Specimens of these three species identified by Distant, a great authority on the taxonomy of the Indian Rhynchota, are available in the collection of the Entomological Section of the Imperial Agricultural Research Institute and are illustrated in Plate XLIV. While working on the *Pyrilla* pest at Pusa during the last two years it was observed that each of the three species named above was most abundant at a particular time of the year, *perpusilla* being most common during April, May and June, *aberrans* from June up to the end of August, and *pusana* from the middle of August onwards. It was also observed that *aberrans* and *pusana* freely copulated with each other. These observations naturally aroused the suspicion that perhaps we were not dealing with three distinct species but with two, or perhaps even one species only. To clear this point a large series of specimens were carefully examined and it was noticed that anatomically as well as in coloration *aberrans* and *pusana* formed the ends of a series having several intermediate specimens. The structure of the male genital appendages in Rhynchota are known to afford very reliable characters for specific differentiation. These organs were studied and found to be identical in *aberrans* (Plate XLVI, fig. 1) and *pusana* (Plate XLV, fig. 2) and slightly different from those of *perpusilla* (Plate XLV, fig. 1). Thus it was evident that at least *aberrans* and *pusana* were actually one and the same species. To confirm this conclusion a number of specimens of the three species were sent to the British Museum for comparison with the type specimens. Mr. W. E. China, the officer in charge of the Rhynchota collections in that museum, very kindly compared my specimens and sent me drawings of the male genitalia of the type specimens available, as the rules of the British Museum do not allow type specimens to be sent out. This reference has revealed an interesting state of affairs. It is evident that the Pusa specimens identified by Distant as *P. aberrans* Kirby distinctly differ from Kirby's type specimen of this species which was described from Ceylon. The type specimen is much smaller in size and its genitalia (Plate XLVI, fig. 2) are different from those of the so-called *P. aberrans* specimen from Pusa (Plate XLVI, fig. 1). Through the kindness of the Entomologists concerned I have examined named specimens of *P. aberrans* in the collections of the Zoological Survey of India, Departments of Agriculture, C. P. and Madras and find that they all resemble Pusa specimens of *P. aberrans* as named by Distant and are therefore not true *P. aberrans*. Thus there is enough justification for the assumption that we have not yet come across true *P. aberrans* Kirby in India and that Distant's *P. aberrans* described from Pusa is a synonym of *P. pusana* Dist., the former being at the most a colour form of the latter.

Regarding *P. perpusilla* Walk. and *P. pusana* Dist., the male genitalia of these two species as stated already differ from each other only slightly (Plate XLV, figs. 1 and 2). It therefore appears that these two forms are varieties of the same species. *P. pusana* Dist. having been described later (1914) will fall as synonym of *P. perpusilla* Walk. (described in 1851) and we will have only one species of *Pyrilla* in India, *viz.*, *P. perpusilla* Walk. But we are not sure whether Distant's identification of this species was correct. The type of *perpusilla* Walk. is a female specimen collected from North Bengal. There is no topo-typical male specimen in the British Museum, nor there is one in the collections of the Indian Museum, Calcutta, or Pusa. For the present these two species will therefore have to be kept distinct.

PLATE XLV

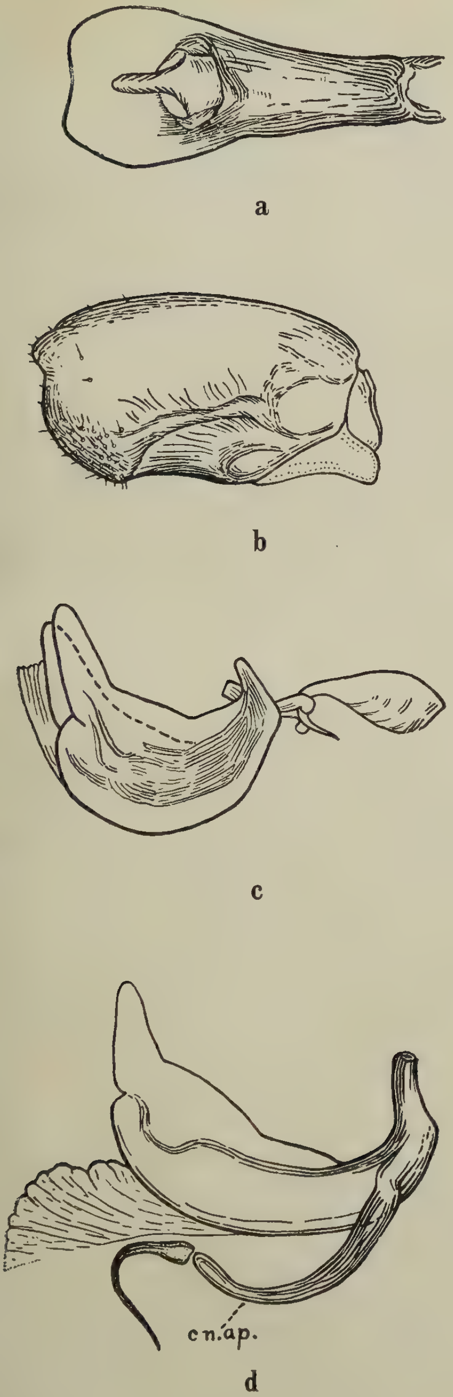


FIG. 1.—Male genitalia of *Pyrilla perpusilla* Walk.
a. Anal tube (×46). **b.** Paramere (×42). **c.** Aedeagus, lateral view (×40). **d.** Aedeagus, medial longitudinal section, showing the conjucntiva appendage (*cn. ap.*).

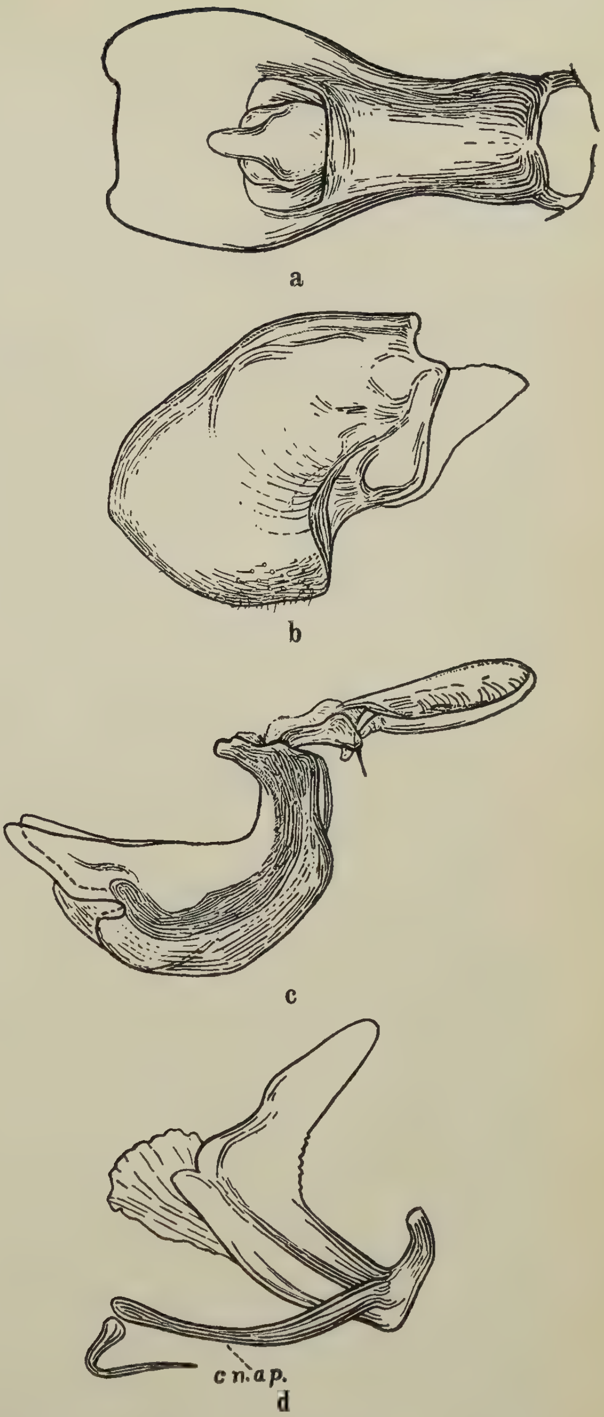


FIG. 2.—Male genitalia of *Pyrilla pusana* Dist.
a. Anal tube, dorsal view (×72). **b.** Paramere (×48). **c.** Aedeagus, lateral view (×48). **d.** Aedeagus, medial longitudinal section, showing the conjunctiva appendage (*cn. ap*).

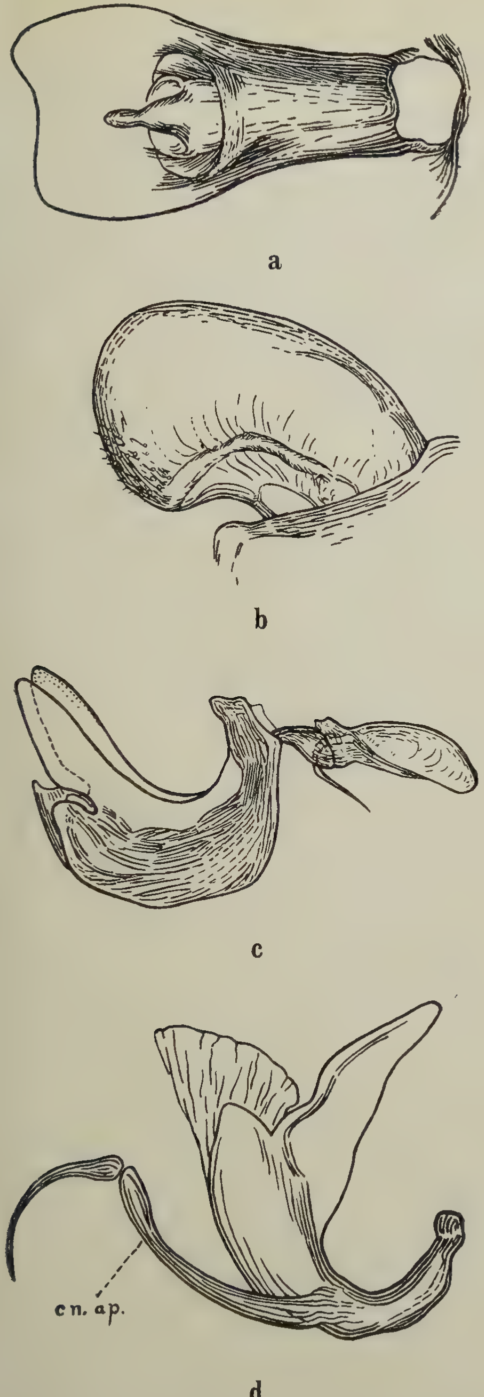


FIG. 1.—Male genitalia of *Pyrilla aberrans* Dist. (nec Kirby)

a Anal tube (×48). **b** Paramere (×47). **c** Aedeagus, lateral view (×50). **d** Aedeagus, medial longitudinal section, showing the conjunctiva appendage (*cn. ap.*)

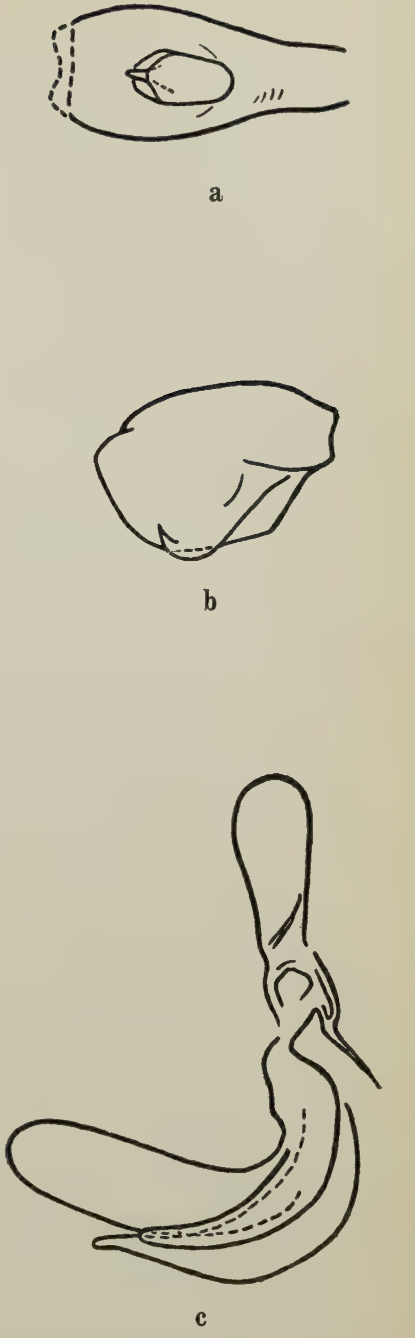


FIG. 2.—Male genitalia of the type specimen of *Pyrilla aberrans* Kirby (supplied by Mr. W. E. China).

a Anal tube. **b** Paramere. **c** Aedeagus, dissected.

The wide difference in growth characters between the two parents—clumps of P O J 213 in front of *Bambusa arundinace* Willd.

SUGARCANE-BAMBOO HYBRIDS

BY

T. S. VENKATRAMAN, C. I. E., B.A., I.A.S.
Government Sugarcane Expert, Imperial Sugarcane Breeding Station, Coimbatore

(Received for publication on 25th May 1937)

(With Plates XLVII and XLVIII)

Encouraged by success in the intergeneric hybridization between sugarcane (*Saccharum officinarum*) and sorghum (*Sorghum Durra* Stapf.) in the year 1930 and since successfully repeated in Florida, Java and Hawaii, the writer of this note has been on the look out for other *genera* to cross with sugarcane. Success in sugarcane breeding depends largely on the extent of variation which it is possible to induce in the seedlings raised ; and it was felt that the bringing in of other *genera* might increase the range of such variation and perhaps also open out new lines for advance.

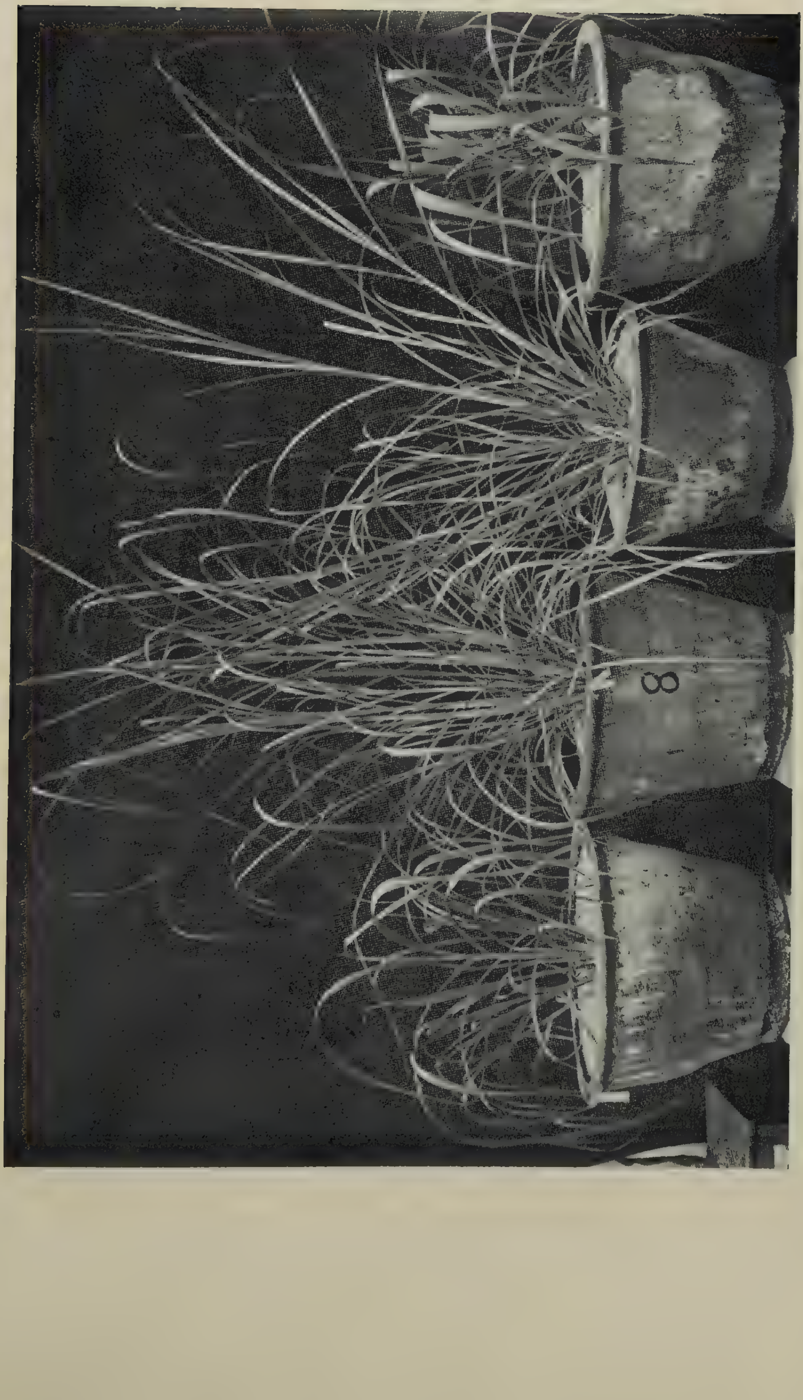
Rows of bamboo clumps, some of them over seventy feet high, constitute an important and useful boundary round the Sugarcane Breeding Station at Coimbatore. Though the differences between the sugarcane and the bamboo, in size of plant, habit of growth , duration of life-cycle and other characters, are marked and obvious —they belong to the two different Sub-families of the Order Gramineae, the Panicoideae and Pooideae respectively—it was felt that there existed, nevertheless, a similarity between them. The attempt, which on a previous occasion was given up, was therefore renewed in December 1936, to effect a cross between the two plants.

On the sugarcane side the parents employed were the two Java canes, POJ 213 and POJ 2725 ; these were the only varieties available for crossing at the time, December being practically the end of the cane flowering season at Coimbatore. Both these canes have little fertile pollen of their own but set seed profusely when cross-pollinated with other cane varieties. POJ 2725 was the cane which was first used in the intergeneric hybridization with sorghum. The bamboo pollen employed was that of *Bambusa arundinacea* Willd. from Madras, the few available inflorescences being railed to Coimbatore in special receptacles. The attempt has yielded twenty-nine hybrids—eighteen with POJ 213 and eleven with POJ 2725, as mother parent.

Though even from the very early stages these hybrids displayed characters not previously noticed in sugarcane seedlings during almost a quarter century of sugarcane hybridization at Coimbatore, this announcement was delayed till the plants showed definite and clear bamboo characters. These have since been found in the mode of underground branching, nature of shoot and bud formation, central cavity in the stem, anatomical characters of the stem and root and number of chromosomes.

It is well known that sugarcane seedlings display considerable variations among themselves in the F_1 generation. The present batch of twenty-nine hybrids show similar variations and different bamboo characters are showing themselves in different plants. They further show abnormalities even more numerous than those met with in the intergeneric cross with sorghum.

PLATE XLVIII



P O J 213 seedling.   Two seedlings of P O J 213 × bamboo.   P O J 213 seedling.

REVIEWS

An Outline of cytological technique for plant breeders. Issued by the Imperial Bureau of Plant Genetics (for Crops other than Herbage), School of Agriculture, Cambridge. Price 1*s*. 6*d*.

THE Imperial Bureaux continue to justify themselves by their works. This small and cheap monograph is one which ought to be in the hands of every plant breeder throughout India. It is also commended to the notice of all University Departments that have to deal with plant cytology. It might well be a practical handbook for advanced students in botany. The following sections from the introduction are worth quoting :—

" Nuclear cytology, or karyology, has in recent times become so intimately linked up with its sister science, genetics, that it is now almost impossible to draw a sharp line of distinction between them, except on a basis of the technique employed. So close is the association that the name cyto-genetics has been suggested for the combined science. Inasmuch as plant breeding represents a practical application of genetics, it is no longer possible even for the strictly practical breeder to escape the influences of cytology. There are, in fact, several plants in which a cytological study has provided the clue to unsolved problems in breeding, as in the potato, the banana and a number of others, and there is no plant in which a knowledge of cytology cannot be said to help materially the plant breeder. It is true of course that plant breeding can be and is often carried on without a cytological investigation of the material, but such are the ramifications of pure and applied science that it would be difficult to say with certainty even in such cases that no help at all has been received from cytology. We might illustrate the value of cytology to the breeder by the following analogy from the arts ; many a blind person has learned to play a musical instrument superlatively well, but the normal person finds his eyes of great assistance in learning and playing. The plant breeder can receive similar assistance, though not perhaps always so directly, from the observations of chromosomes.

" Perhaps the most elementary problem in karyology is chromosome number, and yet a knowledge of this alone can be of great assistance to the breeder when he is dealing with a series of related forms. It is sufficient in this instance to mention the case of wheat ; in the classification of wheats today the primary division is usually based on chromosome number, and this is the first consideration in starting any breeding programme in connexion with the wheat plant. The same is true of potatoes, cotton and many other plants.

"While the mere study of chromosome numbers can help considerably in elucidating the relationships between different forms, a more careful study of the morphology of the somatic chromosomes can help even more. Many important contributions in this direction have been made by Levitsky, though as his work has been published mostly in Russian journals it is not perhaps as well known as it deserves to be. That part of his work which is of interest to plant breeders has been reviewed in *Plant Breeding Abstracts*, and a translation describing his methods of measuring chromosomes is one file at the Bureau. By his carefully developed methods of fixation and measurement he has carried out detailed investigations of morphological characteristics of somatic chromosomes from which interesting conclusions have been drawn regarding the phylogenetic relationships between certain species. The work of Levan, in *Allium*, may also be mentioned here, for it indicates the value of the pollen grain divisions in such studies ; here only the haploid set is present as compared with the diploid set in the more commonly used root-tips. Recent work on the genotype control of chromosome size indicates the need for great caution, however, in interpreting results concerning the dimensions of chromosomes.

"The third line of cytological work, and perhaps the most important, supplying information of value to the geneticist and to the plant breeder, is the study of meiosis in hybrids. The degree of regularity encountered here is the important factor in determining the fertility of the hybrid and hence the ultimate chance of success from a given combination of parents. But the more detailed study of meiosis can tell us more than this. For instance, we know that chiasmata and genetical crossing-over are intimately related phenomena, in fact, on the basis of the now widely accepted partial chiasmatypy hypothesis, they are different aspects of the same phenomenon. It follows, therefore, that the observation of chiasma formation between the chromosomes of different parents at the first division of meiosis will shew whether it is possible to transfer characters from one species to the other as the near-immune reaction to rust was transferred from a *durum* to a *vulgare* wheat to produce Hope and H-44 as characters from Asiatic cottons were transferred to New World cottons by Harland. Again, the observation of restitution nuclei will indicate the possibility of obtaining 'amphidiploid' forms : the studies of pairing in hybrid wheats have elucidated the genetical relationships between the different forms of wheat and related genera and shewn the possibility of utilizing the latter in producing entirely new forms of cereals. This is not the place to discuss all the applications of cytology to plant breeding, but we may confidently assert that in the future the microscope will become one of the plant breeder's most important tools, and that plant breeding will benefit immeasurably thereby."

The methods described can be found in various text-books in more or less detail but this monograph gives the main points of the paraffin method, the smear method, staining, microscopic technique and drawing, along with a very useful set of formulae for fixatives and ends up with a small and useful bibliography. The material has been prepared by Mr. J. L. Fyfe and the manuscript has been read by that well-known cytologist, Dr. C. D. Darlington, while Mr. H. W. Howard has given assistance in a number of details. The price of the monograph is 1*s*. 6*d*. Copies can be obtained from the Deputy Director of Plant Genetics, School of Agriculture, Cambridge, England [W. B.].

Vegetative propagation of tropical and sub-tropical fruits. By G. St. Cl. Fielden and R. J. Garner. (*Tech. Comm. No. 7 of the Imperial Bureau of Fruit Production, East Malling, Kent, England, 1936*). Pp. 67, bibl. 123. Price 2*s*.

This compilation forms the second of a series of articles issued by the Imperial Bureau of Fruit Production on the vegetative propagation of tropical and sub-tropical horticultural crops, the first on citrus having appeared in 1932.

It is based on an examination of existing literature and on the answers to enquiries sent to many workers in the tropics. All the same it would appear that there are a considerable number of sources particularly in departmental bulletins and agricultural journals which still remain to be tapped. The section on methods of vegetative propagation (pages 7 to 17) is very useful and the drawings of various types of grafts, some of which will be new to several readers, are clear and self-explanatory. Those who have good hands for this type of work will find delight in experimenting with the many kinds of grafting and budding here indicated. There are certain tropical substitutes for such things as raffia and grafting wax that might possibly have been mentioned. For example, the fibrous layer inside the stalk petiole of the banana leaf makes very excellent material known as *sopat* in the Bombay-Deccan for tying on buds. Grafting wax is in various places in India replaced by a mixture of mud and cow-dung which is often quite effective. The reviewer would draw special attention to the section of etiolating methods on page 10 which is well worth a very extended trial in the tropics, particularly on account of the necessity of getting stocks of uniform character for really effective horticultural experiments in the future.

The second part of the publication deals with about 100 types of fruit and in each case mentions methods of vegetative propagation that have been found successful or are under experiment. In addition to the ordinary well-known fruit crops, there are also a considerable number of plants which are more or less wild, such as the *Spondias* species, the *Dillenia* species and the *Carissa* species. But these should certainly receive attention as from them may develop really valuable fruit varieties. In this connection special mention should be made of *Zizyphus* to which the last section is devoted. The method of budding found most successful in the Bombay-Deccan has not been mentioned here. This consists in decapitating the stock, removing the bark from the top 2 inches and sliding over it a complete unbroken ring of bark from a similar sized scion containing the bud. *Zizyphus* certainly has considerable possibilities of development. The mango (pages 42-45) naturally comes in for a good deal of attention. Some of the interesting points mentioned are :—

P. 43—the inducing of a flush of growth by giving one pint of one-ounce-to-the-gallon solution of nitrate of soda about a week before grafting to the stock.

P. 44—the possibility of utilising etiolation.

In conclusion, the reviewer would like to point out the desirability in future research work on vegetative propagation of going in some detail into the anatomy of the grafting union somewhat in the manner in which this was done in Sorauer's book on Plant Physiology [W. B.].

Horticultural aspects of woolly aphis control together with a survey of the literature. By R. M. Greenslade. (*Tech. Comm. No. 8 of the Imperial Bureau of Fruit Production, East Malling, Kent, England, 1936*). Pp. 88, bibls. 555 (general) and 156 (biologic control). Price 2*s*. 6*d*.

The voluminous literature published during the last hundred years on woolly aphis or American blight and the divergence of opinion there expressed have long demanded examination and critical analysis.

In November 1933 a Memorandum and Questionnaire on the incidence and control of the pest was circulated by the Imperial Bureau of Fruit Production to all the apple-growing countries of the world and co-operation invited.

The literature and the replies to the questionnaire have been thoroughly sifted by the present author, who has now for some years at East Malling been testing new seedlings for immunity, investigating control measures in the orchard and studying the causes of immunity or resistance, and is, therefore, in an exceptionally good position for examining the situation. This he does most clearly and concisely for practical horticulturists no less than for fellow investigators in *Technical Communication* 8.

First the insect and its habits, its spread in the orchard, its methods of feeding and its possible alternative hosts are dealt with. Next the bearing of climatic factors, temperature, humidity, wind and sunlight on its incidence is considered. In many parts of the Empire the normal combination of these factors is propitious for a heavy attack, whereas in England an increase in temperature and sun and in Rumania an increased rainfall will induce it. Control measures are considered in detail :—(1) Artificial control including spraying, fumigation, tree injection, cultural practice, etc., (2) Natural control by *Aphelinus mali* and other parasites.

The varying measure of success achieved in different countries is noted. (3) Control by use of resistant stocks and varieties. A particularly interesting account is given of existing resistant varieties, of the breeding work in progress in England at Merton and East Malling and of the few indications afforded as yet of the possible causes of resistance. Lastly (4) Control by legislation, *e.g.*, quarantine measures, etc.

The literature from nearly 300 sources is further dealt with in the two annotated bibliographies which follow. The first, general, contains 555 references, while the second contains 156 references to articles on the biologic control of the pest.

Finally the Memorandum and Questionnaire noted previously are reproduced in full, together with a list of persons who replied. This list forms, incidentally, a useful index of workers interested in the subject.

Both to investigators and growers this publication should prove of great reference value.

Een Bijdrage Tot DeKennis Omtrent Het Bloeien En Het Vruchtdragende Vermogen Van Den Mangga (*Mangifera indica* **L.**). **(A contribution to the knowledge of the flowering and fruiting habits of the mango tree**, *Mangifera indica* **L.**). By A. P. C. BIJHOUWER. (H. Veenmann and Zonen—Wageningen, Holland).

THIS important paper is printed in Dutch and is issued as a separate small volume. There is a very useful English summary of six pages. The writer deals with the morphology and physiology of flowers and flower-heads and describes his experiments on caging (in connection with pollination) and on bees. It would appear that bee-keeping associated with a mango orchard is likely to be a useful side-line [W. B.].

RECENT PUBLICATIONS OF THE IMPERIAL AGRICULTURAL BUREAUX

I. OBTAINABLE FROM THE IMPERIAL BUREAU OF SOIL SCIENCE, ROTHAMSTED EXPERIMENTAL STATION, HARPENDEN, HERTS

Periodical Abstracts

Lists of publications and papers on Soil Science published in the Empire Overseas in :—

	Price	
	s.	*d.*
1933	1	0
1934	1	0
Soil Research in the British Empire published during 1935	1	0
Lists of Publications relating to Soils and Fertilisers—		
Published monthly, per annum, post free	10	0
Monthly Letters—		
Free to recipients, within the British Empire, of 'Publications relating to Soils and Fertilisers.' Subscription, outside the Empire, per annum	4	0
Recent Developments in Soil Analysis—		
Quarterly Supplement to the above publications. Separate copies each	0	6

Occasional Papers

Technical Communications—		
34. Tropical Soils in relation to Tropical Crops	2	6
Annual Report : For the year 1934-35	0	6
Bibliographies—		
Bibliography on Coffee	2	0
Catalogue of Journals and Periodicals in the Library of Rothamsted Experimental Station	2	0
Special Publication—		
The Katamorphism of Igneous Rocks under Humid Tropical Conditions (by the late Sir J. B. Harrison)	5	0
Bibliography of Soil Science, Fertilizers and General Agronomy, 1931-34	25	0

II. OBTAINABLE FROM THE IMPERIAL BUREAU OF ANIMAL HEALTH, VETERINARY RESEARCH LABORATORY, NEW HAW, WEYBRIDGE, SURREY

Abstracting Journal

The Veterinary Bulletin—		
1931. Vol. 1. Quarterly (1st Number, April)	7	6
Annual Subscription	20	0
Subsequent Volumes. Monthly (1st Number, January)	5	0
Annual Subscription (postage paid)	40	0

Indexing Publication

	Price s.	d.
Index Veterinarius—Four issues a year. First issue April 1933— Annual Subscription (postage paid). Volumes I to III mimeographed, Volume IV onwards printed	100	0

III. OBTAINABLE FROM THE IMPERIAL BUREAU OF ANIMAL NUTRITION, ROWETT RESEARCH INSTITUTE, BUCKSBURN, ABERDEEN

Journal

	s.	d.
Nutrition Abstracts and Reviews. (Issued under the direction of the Imperial Agricultural Bureaux Council, the Medical Research Council and the Reid Library)—		
Subscription per volume of 4 numbers	42	0
Per single number	13	0

Occasional Papers

	s.	d.
Technical Communications—		
7. Wheat : Pre-eminence as a Cereal Food, Nutritive Value ; Relation to Health and Disease	1	0
Occasional Communications—		
1. The Effect of Climate on the Composition of Pasture Plants		

IV. OBTAINABLE FROM THE IMPERIAL BUREAU OF PLANT GENETICS (FOR CROPS OTHER THAN HERBAGE), PLANT BREEDING INSTITUTE, SCHOOL OF AGRICULTURE, CAMBRIDGE

Journal

	s.	d.
Plant Breeding Abstracts—		
Quarterly. Annual Subscription	15	0
Single copies	5	0

Occasional Papers

	s.	d.
Indexes to Plant Breeding Abstracts—		
Subject Index to Vols. I to V of Plant Breeding Abstracts	5	0
Supplements to Plant Breeding Abstracts—		
Summary of Reports received from Countries exclusive of the British Empire, 1928-31. Supplement I	2	6
Summary of Reports received from Stations in the British Empire, 1932-1935. Supplement II	5	0
Technical Communications—		
Vernalization and Phasic Development of Plants (Joint Publication of the Imperial Bureaux of Plant Genetics)	10	0
Bibliographical Monographs—		
The Experimental Production of Haploids and Polyploids	5	0
Breeding Resistant Varieties, 1930-33 (Supplement)	2	0
An Outline of Cytological Technique for Plant Breeders	1	6

V. Obtainable from the Imperial Bureau of Plant Genetics (Herbage Plants), Welsh Plant Breeding Station, Agricultural Buildings, Alexandra Road, Aberystwyth, Wales

Journals

	Price *s*	*d*
Herbage Abstracts—		
Quarterly. Annual Subscription	15	0
Single numbers	5	0

Herbage Reviews—

Subscription is at present—Vol. 1 (1933), Vol. 2 (1934), Vol. 3 (1935), Vol. 4 (1936)—included in that to Herbage Abstracts

Occasional Papers

	s	*d*
Bulletins—		
17. Vernalization and Phasic Development of Plants. December, 1935 (Joint publication of the Imperial Bureaux of Plant Genetics)	10	0

VI. Obtainable from the Imperial Bureau of Fruit Production, East Malling Research Station, East Malling, Kent

Journal

Horticultural Abstracts—

A quarterly abstract publication of current horticultural literature—

	s	*d*
Annual Subscription	15	0
Single copy	4	0

Technical Communications

	s	*d*
8. Horticultural Aspects of Woolly Aphis Control together with a Survey of the Literature R. M. Greenslade	2	6

Occasional Papers

	s	*d*
3. Annotated Bibliography on Bitter-Pit. 1934	1	6
Other Publications—		
Index to Volumes I-X of the *Journal of Pomology and Horticultural Science*, 1933. Compiled by Bureau, published by the Editors of the *Journal of Pomology and Horticultural Science*. Available from the Bureau	5	0
Old and New standpoints on senile degeneration, 1931. By A. P. C. Bijhouwer	0	6

VII. Obtainable from the Imperial Bureau of Animal Genetics, Institute of Animal Genetics, University of Edinburgh, King's Buildings, West Mains Road, Edinburgh

Journal

	s	*d*
Animal Breeding Abstracts (Quarterly) Commencing April, 1933. Annual Subscription	15	0

Occasional Papers

	Price *s*	 *d*
Animal Breeding in the British Empire. A Survey of Research and Experiment, 1934	2	0

VIII. OBTAINABLE FROM THE IMPERIAL BUREAU OF AGRICULTURAL PARASITOLOGY, INSTITUTE OF AGRICULTURAL PARASITOLOGY, WINCHES FARM DRIVE, HATFIELD ROAD, ST. ALBANS, HERTS

Journals

	s	*d*
Bibliography of Helminthology. For the year 1933—		
Library Edition, bound in cloth	10	6
Stiff paper cover only	8	0
Helminthological Abstracts. (Issued in five or six parts per volume, each volume, covering the literature of a single year.) Subscription per volume, payable in advance	30	0
Completed volumes bound in cloth	32	0

Occasional Papers

	s	*d*
The Effects of some Natural Factors on the Second Ecdysis of Nematode Infective Larvae. By G. Lapage. 25 pp.	4	0
Notes and Memoranda—		
11. Recent Developments in the Control of *Heterodera marioni*. 6 pp.	1	6

PUBLICATIONS ISSUED BY THE IMPERIAL INSTITUTE OF ENTOMOLOGY, 41, QUEEN'S GATE, LONDON, S. W. 7

Bulletin of Entomological Research.—Published quarterly and containing original articles on Economic Entomology. Issued post-free.

	s	*d*
Annual subscription (payable in advance)	30	0

Review of Applied Entomology.—Consisting of abstracts or reviews of all current literature on Economic Entomology throughout the world. Published monthly in two series. Series 'A' dealing with Insect and other Arthropod pests of cultivated plants, forest trees and stored products of animal and vegetable origin, Series 'B' dealing with insects, ticks, etc., conveying disease or otherwise injurious to man and animals. Issued post-free.

	Series 'A' *s*	 'B' *s*
Annual subscription (payable in advance)	30	15
Vol. XXIII (1935)	42	21

	Price	
	s	*d*
Zoological Record—Part Insecta.—Published annually about July in each year and containing as complete a record as possible of the literature of the previous year chiefly from the systematic standpoint.		
Annual subscription (including postage)	15	6
Report of the Fourth Imperial Entomological Conference, 19-27th September 1935	4	0

PUBLICATIONS OBTAINABLE FROM THE IMPERIAL MYCOLOGICAL INSTITUTE, KEW, SURREY

Journal

Review of Applied Mycology.—Annual subscription, 12 monthly parts, with title page and index (post-free)	21	0
Single part	2	0
Title page and index	3	0
Report on the Third Imperial Mycological Conference, 1934	2	0

PUBLICATIONS OF THE IMPERIAL COUNCIL OF AGRICULTURAL RESEARCH, INDIA

---

1. Agriculture and Live-stock in India

A bi-monthly Journal of Agriculture and Animal Husbandry for the general reader interested in Agriculture or Live-stock in India or the Tropics. (Established 1931. Published in January, March, May, July, September and November. Prepayable subscription Rs. 6 or 9*s*. 9*d*. per annum inclusive of Indian postage. Price per part Rs. 2 or 3*s*. 6*d*. inclusive of Indian postage). Volumes I to VI complete are available.

2. The Indian Journal of Agricultural Science

A bi-monthly Scientific Journal of Agriculture and the allied sciences, mainly devoted to the publication of the results of original research and field experiments. (Established 1931. Published in February, April, June, August, October and December. Prepayable subscription Rs. 15 or 24*s*. per annum inclusive of Indian postage. Price per part Rs. 3 or 5*s*. 3*d*. inclusive of Indian postage.) Volumes I to VI complete are available.

3. The Indian Journal of Veterinary Science and Animal Husbandry

A quarterly Journal for the publication of scientific matter relating to the health, nutrition and breeding of live-stock. (Established 1931. Published in March, June, September and December. Prepayable subscription Rs. 6 or 9*s*. 9*d*. inclusive of Indian postage. Price per part Rs. 2 or 3*s*. 6*d*. inclusive of Indian postage.) Volumes I to VI complete are available.

4. Scientific Monographs of the Imperial Council of Agricultural Research

NCAG—SM/1	No. 1. The Fungi of India, By E. J. Butler, C.I.E., D.Sc., M.B., F.R.S and G. R. Bisby, Ph.D. (1931). Price Rs. 6-12-0 or 11*s*. (As. 8.)
NCAG—SM/2	No. 2. Life-histories of Indian Microlepidoptera, Second Series : Alucitidae (Pterophoridae, Tortricina and Gelechiadae), By T. Bainbrigge Fletcher, R.N., F.L.S., F.E.S., F.Z.S. (1932). Price Rs. 3-4-0 or 5*s*. 6*d*. (As. 6.)
NCAG—SM/3	No. 3. The Open Pan System of White- Sugar Manufacture, By R. C. Srivastava, B.Sc. (1935), (2nd edition). Price Rs. 3-2-0 or 5*s*. 6*d*. (As. 9.)
NCAG—SM/4	No. 4. Life-histories of Indian Microlepidoptera : Cosmopterygidae to Neopseustidae, By T. Bainbrigge Fletcher, R.N., F.L.S., F.E.S. F.Z.S. (1933). Price Rs. 4-8-0 or 7*s*. 6*d*. (As. 8.)
NCAG—SM/5	No. 5. The Bombay Grasses, By E. J. Blatter, S.J., Ph.D., F.L.S., and C. McCann, F.L.S. Illustrated by R. K. Bhide. Price Rs. 20-12-0 or 32*s*. 6*d*.
NCAG—SM/6	No. 6. Helminth Parasites of the Domesticated Animals in India, by G. D. Bhalerao, M.Sc. Price Rs. 7-12-0 or 13*s*. 3*d*.
NCAG—SM/7	No. 7. Influence of Manures on the Wilt Disease of *Cajanus indicus* Spreng. and the Isolation of Types Resistant to the Disease. By W. McRae, M.A., D.Sc. (Edin.), F.L.S. and F. J. F. Shaw, D.Sc. (Lond.), A.R.C.S., F.L.S. (1933). Price Rs. 2-4-0 or 4*s*. 3*d*. (As. 5.)

NCAG—SM/8	No. 8. The Silk Industry of Japan with Notes on Observations in the United States of America, England, France and Italy, By C. C. Ghosh, B.A., F.E.S. (1933). Price Rs. 4 or 6*s*. 9*d*. (As. 7.)
NCAG—SM/9	No. 9. Mechanical Cultivation in India. A History of the Large Scale Experiments carried out by Burmah-Shell Oil Storage and Distributing Company of India, Ltd., By C. P. G. Wade. Price Rs. 3-14-0 or 6*s*. 6*d*.
NCAR—SM/10	No. 10. Spotted Boll-worms of Cotton (*Earias fabia* Stoll and *Earias insulana* Boid.) in South Gujarat, (Final report on investigations financed by the Indian Central Cotton Committee. 1923-1931), By B. P. Deshpande, M.Ag. and N. T. Nandkarny. Price Rs. 5-14-0 or 9*s*. 6*d*.
	No. 11. Investigations on the Course and Distribution of the Nerve supplying Levator anguli scapuli and Rhomboideus Muscles and the Formation of the Phrenic Nerve in the Ox, with Observations on certain Anatomical Deviations, by H. N. Chelva Ayyangar, G.M.V.C. (*In the Press*).

5. Miscellaneous Bulletins of the Imperial Council of Agricultural Research

NCAG—MB/1	No. 1. List of Publications on Indian Entomology (1930), Compiled by the Imperial Entomologist, Pusa. Price As. 14 or 1*s*. 6*d*.
NCAG—MB/2	No. 2. List of Publications on Indian Entomology (1931), Compiled by the Imperial Entomologist, Pusa. Price As. 8 or 10*d*.
NCAG—MB/3	No. 3. List of Publications on Indian Entomology (1932), Compiled by the Imperial Entomologist, Pusa. Price As. 12 or 1*s*. 3*d*.
NCAG—MB/4	No. 4. Host Plant Index of Indo-Ceylonese Coccidae, By S. Ramachandran, L.Ag. and T. V. Ramakrishna Ayyar, B.A., Ph.D., F.Z.S. Price Re. 1-10 or 2*s*. 9*d*.
NCAG—MB/5	No. 5. List of Publications on Indian Entomology (1933), Compiled by the Officiating Imperial Entomologist, Pusa. Price As. 9 or 1*s*.
NCAG—MB/6	No. 6. Bee-keeping, By C. C. Ghosh, B.A., F.E.S. (3rd Edition.) Price Re. 1-14 or 3*s*. 3*d*.
NCAG—MB/7	No. 7. List of Publications on Indian Entomology (1934), Compiled by the Officiating Imperial Entomologist and the Imperial Entomologist, Pusa. Price Rs. 1-2-0 or 2*s*.
NCAR—MB/8	No. 8. Selected Clinical Articles, by M. G. K. Sharma, G. P. V. C. and R. L. Kaura, B.V.Sc., M.R.C.V.S. Price As. 8 or 10*d*.
NCAR—MB/9	No. 9. Statistical Methods and their Application to Agronomy : A Bibliography, by K. K. Guha Roy, B.A. Price Rs. 2-2 or 4*s*.
NCAR—MB/10	No. 10. Diseases of Sugarcane and Methods for their Control, by L. S. Subramaniam. Price Re. 1-14-0 or 3*s*. 3*d*.
NCAR—MB/11	No. 11. Tables of Standard Errors of Mendelian Ratios, By Swarn Singh Purewal, and P. Krishna Rao. Price As. 12 or 1*s*. 3*d*.
NCAR—MB/12	No. 12. List of Publications on the Botany of Indian Crops, Part II, for the period 1928-32, by R. D. Bose. Price Rs. 3-6-0 or 5*s*. 9*d*.
NCAR—MB/13	No. 13. Two New Statistical Tables based upon Fisher's '*t*', by M. Vaidyanathan. Price As. 6 or 8*d*.
NCAR—MB/14	No. 14.—List of Publications on Indian Entomology (1935), Compiled by the Imperial Entomologist. Price Re. 1-4-0 or 2*s*.

6. Annual Report of the Imperial Council of Agricultural Research

NCAG—AR/31	Annual Report of the Imperial Council of Agricultural Research for the years 1929-30 and 1930-31. Price As. 12 or 1s. 3*d*. (Anna 1).
NCAG—AR/32	Annual Report of the Imperial Council of Agricultural Research for the year 1931-32. Price As. 6 or 8*d*. (As. 2).
NCAG—AR/33	Annual Report of the Imperial Council of Agricultural Research for the year 1932-33. Price As. 6 or 8*d*.
NCAG—AR/34	Annual Report of the Imperial Council of Agricultural Research for the year 1933-34.
NCAG—AR/35	Annual Report of the Imperial Council of Agricultural Research for the year 1934-35.
NCAR—AR/36	Annual Report of the Imperial Council of Agricultural Research for the year 1935-36.

7. Agriculture and Animal Husbandry in India (formerly called Review of Agricultural Operations in India)

NCAG—R. 1/29	Review of Agricultural Operations in India, 1928-29. Price Rs. 3-2-0 or 5*s*. 6*d*. (As. 8).
NCAG—R. 1/31	Review of Agricultural Operations in India, 1929-31. Price Rs. 5 or 8*s*. 3*d*. (As. 10).
NCAG—R. 1/33	Review of Agricultural Operations in India, 1931-33. Price Rs. 5-12-0 or 9*s*. 6*d*.
NCAG—R. 1/35	Agriculture and Animal Husbandry in India : Part I—Crop Production. Price Re. 4-14-0 or 8*s*. Part II—Animal Husbandry. Price Re. 1 or 1*s*. 9*d*.

8. Proceedings of the Board of Agriculture and Animal Husbandry

NCAG—P. 1	Proceedings of the First Meeting of the Animal Husbandry Wing of the Board of Agriculture and Animal Husbandry held at New Delhi from the 20th to 23rd February 1933, with Appendices. Price Rs. 5-14-0 or 9*s*. 6*d*.
NCAR—P. 2	Proceedings of the First Meeting of the Crops and Soils Wing of the Board of Agriculture and Animal Husbandry in India held at Delhi from the 25th February to the 2nd March 1935, with Appendices. Price Rs. 6 or 9*s*. 9*d*.

A Description of the Imperial Institute of Veterinary Research, Muktesar, and its Sub-station, the Imperial Veterinary Serum Institute, Izatnagar, by F. Ware, F.R.C.V.S. Price Re. 1-4-0 or 2*s*.

The Production of Cigarette Tobacco by Flue-curing, by F. J. F. Shaw, C.I.E., D.Sc., A.R.C.S., F.L.S. and Kashi Ram. *Imp. Inst. Agri. Res. Pusa Bull.* No. 187. Reprinted (1935). Price Re. 1 or 1*s*. 9*d*.

A Handbook of Statistics for Use in Plant Breeding and Agricultural Problems, By F. J. F. Shaw, C.I.E., D.Sc., A.R.C.S., F.L.S. Price Rs. 4-6-0 or 7*s*. 3*d*.

[Copies of the above publications can be had from :—

The Manager of Publications, Civil Lines, Delhi, India.

In Europe and America.—From Public Department (Publications Branch), Office of the High Commissioner for India, India House, Aldwych, London, W. C. 2.]

ERRATA

The Indian Journal of Agricultural Science

Vol. 6, Part VI, December 1936.

Page 1257, line 6, *for* ' on page 12 ' *read* ' on page 1252 '.

Page 1260, line 5 from bottom, *for* ' flowering duration—figures ' *read* ' flowering-duration-figures '.

Page 1272, line 9, *for* ' in paragraph 6 of ' *read* ' in paragraph 6 '.

Vol. 7, Part I, February 1937.

Page 139, statement, column 1, *add* 8 **PpTtEe** below 4 **PpTtEE**.

Page 44, line 1, *for* 'f ' *read* ' if '.

Page 44, paragraph 7, line 2, *for* ' **PpTtee** ' *read* ' **ppTtee** '.

GIPD S3—759 IC AR—12-7-37—800.

ORIGINAL ARTICLES

STUDIES IN PLANT BREEDING TECHNIQUE

II. THE DESIGN OF FIELD TESTS OF PLANT BREEDING MATERIAL

BY
J. B. HUTCHINSON
AND
V. G. PANSE
Institute of Plant Industry, Indore, Central India

(Received for publication on 13th February 1937)

(With five text-figures)

CONTENTS

	PAGE
I. INTRODUCTION	531
II. METHODS	532
(*a*) Simple replication and randomisation	532
(*b*) Compact family blocks	537
(*c*) Partial analysis	544
(*d*) Efficient utilisation of limited seed	549
(*e*) Small bulk trials	555
III. DISCUSSION	557
IV. SUMMARY	562
V. ACKNOWLEDGMENTS	563
REFERENCES	563
APPENDIX	564

I. INTRODUCTION

IN the first paper in this series [Hutchinson and Kubersingh, 1936] an analysis was made of the efficiency of the current method of progeny row breeding for the improvement of cotton. It was there shown that environmental effects contributed so much to the variability of the material that a very large part of the genetic differences present remained undetected. The belief that current methods were inefficient and the necessity for deciding whether further selection in existing improved strains would be profitable led to an attempt in the 1934-35 season to determine the amount of genetic variability remaining in two strains of Malvi cotton. For this purpose the principles of replication and randomisation, which form the basis of modern methods of agricultural experimentation, were adopted.

The success of the method, in spite of the extremely small plot size necessary, led to extensive development, and it is proposed to present an account of its working, and of the modifications which have been devised to increase its efficiency.

Two difficulties appear to have dissuaded plant breeders from trying out these methods earlier. The first is that it did not seem possible to employ a replicated layout with the very small amounts of seed available from single plants. The only answer to this is that even with extremely small plots the error variances are not unreasonably high. The second is the very real one that in the early stages of breeding work, the material available for test may be very heterozygous. Genetic variability will, of course, contribute to the error variance, and where there are no great differences in variability between progenies, there will be no risk of drawing erroneous conclusions. On the other hand, the estimation of genetic variance is a matter of first importance to the plant breeder, and for this reason the method of replication and randomisation is of value as much where there is reason to believe that the material may be heterogeneous in genetic variance as where it is homogeneous.

II. Methods

(*a*) *Simple replication and randomisation*

Replication of progeny rows was first tried out on two selected strains of Malvi cotton, Malvi 1 and Malvi 9. Examination of the yield data from the 1933-34 crop showed that it was only possible to rely on obtaining 150 sound selfed seeds from a plant from which it was desired to raise a progeny. As it is necessary at Indore to sow at least three seeds per hole to ensure a satisfactory stand, the design was based on fifty holes per progeny. From experience of a uniformity test with cotton [Hutchinson and Panse, 1935, 1] it was clear that it would be advantageous to divide the progenies into as many plots as was reasonably possible. It was therefore decided to lay out each experiment in ten randomised blocks. Each plot consisted of a single row of five holes. The spacing employed was the same as had been used in non-replicated progeny row work, namely one ft. between holes and two ft. between rows.

In the Malvi 1 trial progenies were grown from ten sister plants (ten plots per block). In the Malvi 9 trial, progenies were grown from ten sister plants in each of five families, giving fifty plots per block. Two rows were sown all round each block with bulk seed from discarded plants of the same strain in order to equalise competition effects. Diagrams of the layouts of the two experiments are given in Figs. 1 and 2.

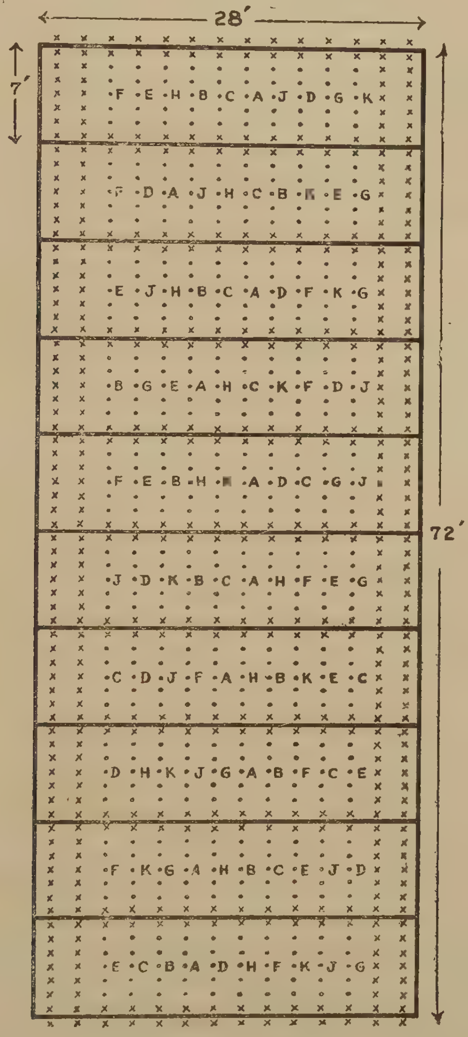

28′
7′
72′
F E H B C A J D G K
F D A J H C B K E G
E J H B C A D F K G
B G E A H C K F D J
F E B H K A D C G J
J D K B C A H F E G
C D J F A H B K E C
D H K J G A B F C E
F K G A H B C E J D
E C B A D H F K J G

FIG. 1. Field plan of replicated progeny row trial (Malvi 1, 1934).

Experimental plots ....

Guard rows ××××

Ten progenies *A—J*

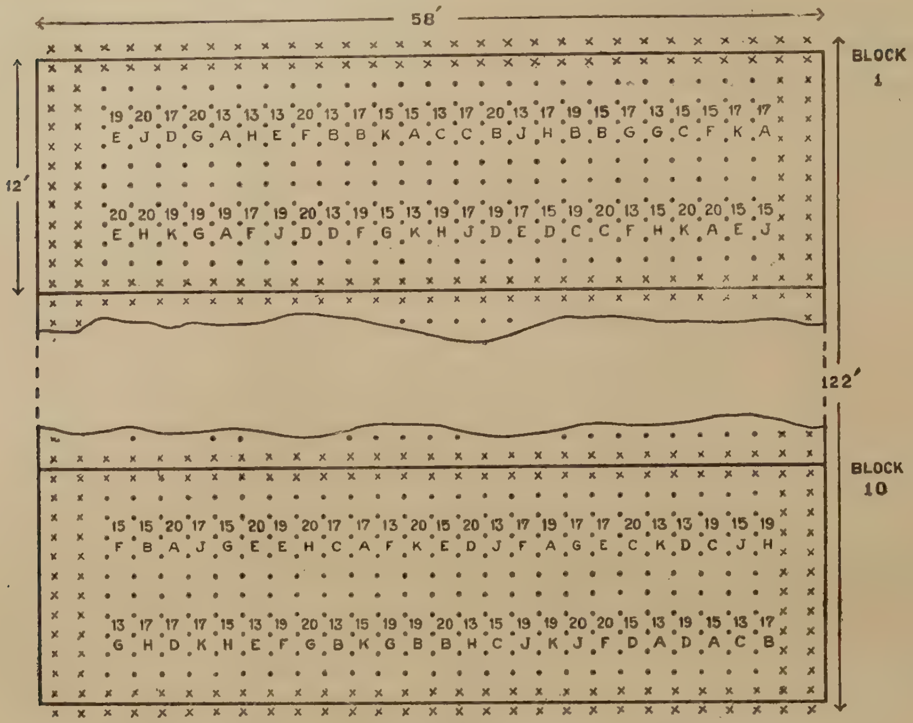

58′
BLOCK 1
12′
19 20 17 20 13 13 13 20 13 17 15 15 13 17 20 13 17 19 15 17 13 15 15 17 17
E J D G A H E F B B K A C C B J H B B G G C F K A
20 20 19 19 19 17 19 20 13 19 15 13 19 17 19 17 15 19 20 13 15 20 20 15 15
E H K G A F J D D F G K H J D E D C C F H K A E J
122′
BLOCK 10
15 15 20 17 15 20 19 20 17 17 13 20 15 20 13 17 19 17 17 20 13 13 19 15 19
F B A J G E E H C A F K E D J F A G E C K D C J H
13 17 17 17 15 13 19 20 13 15 19 19 20 13 15 19 19 20 20 15 13 19 15 13 17
G H D K H E F G B K G B B H C J K J F D A D A C B

Fig. 2. Field plan of replicated progeny row trial (Malvi 9, 1934).
Five families, 13, 15, 17, 19, 20.
Experimental plots . . . .
Guard rows × × × × ×
Ten progenies (*A*—*J*) in each family.

After germination, seedlings were thinned to one per hole. A count of the stand per plot was made at first picking. Losses were due almost entirely to failures in germination and deaths from wilt. Analyses of variance were carried out on the stand figures and provided evidence on the importance of progeny differences in these two characters. (In subsequent trials germination and stand after thinning were recorded, in order that the effects of differences in germinating power and differences in wilt-resistance might be separately estimated). With such extremely small plots chance fluctuations in stand may be expected to exercise a very large effect upon plot yield, and in studying the latter it is necessary to carry out the analysis of variance and then to adjust by the analysis of covariance of yield on stand.

In Tables I and II are summarised analyses of variance of stand, crude and adjusted yield, staple-length, and ginning percentage for the two 1934-35 experiments.

TABLE I

Summary of analyses of variance of representative important characters in Malvi 9, 1934-35

Due to differences between	Degrees of freedom	Mean squares				
		Stand	Crude yield	Adjusted yield	Staple length	Ginning percentage
Blocks . . .	9	1·13	801·2	. .	11·99	13·81
Families . . .	4	4·19	5378·1	2817·2	10·72	7·83
Progenies within families	45	1·82	412·1	280·8	1·32	2·76
Remainder (=error) .	439*	0·56	366·7	294·8**	1·04	3·94
Standard error per cent—						
Family comparisons (mean of 100 plots).	. .	3·5	4·0	3·6	0·5	0·6
Progeny comparisons (mean of ten plots).	. .	5·3	13·0	11·5	1·5	1·9

* Reduced by two owing to calculation of values for two missing plots.

** Degrees of freedom reduced by one for the regression component.

TABLE II

Summary of analyses of variance of representative important characters in Malvi 1, 1934-35

Due to differences between	Degrees of freedom	Mean squares				
		Stand	Crude yield	Adjusted yield	Staple length	Ginning percentage
Blocks . . .	9	3·19	1188·3	. .	0·78	32·65
Progenies . . .	9	2·11	446·7	395·2	0·74	24·38
Remainder (=error) .	81	0·87	744·7	417·7*	0·51	2·66
Standard error per cent (mean of 10 plots).	. .	7·2	14·9	11·2	1·0	1·8

* Degrees of freedom reduced by one for the regression component.

It will be seen that the percentage standard errors are very similar in the two experiments, in spite of the fact that the block size was five times as great for Malvi 9 as for Malvi 1. The standard errors fall into two groups :—(1) staple-length and ginning percentage, one to two per cent ; and (2) stand, crude yield and adjusted yield, five to fifteen per cent. These groupings are as might have been expected from consideration of the nature of the characters. Staple-length and ginning percentage are known to be only slightly affected by environmental fluctuations, the full force of which is borne by such characters as stand and yield. It follows that comparisons between progenies are very efficient for lint length and ginning percentage, while for stand and yield, differences of the order of thirty per cent will be reliable. Even so, a level of accuracy high enough to make significant differences in yield of about thirty per cent between progenies is a great advance in plant breeding technique. Between progenies of Malvi 9 from the present data there were significant differences in stand, and of Malvi 1 in stand and ginning percentage.

In Malvi 9, the fifty progenies were taken ten from each of five families, so that it is possible to make a further comparison between family means. For this comparison, the standard error per cent will be $1/\sqrt{10}$ times that for progenies (Table 1). Stand and yield comparisons are now sufficiently accurate to detect with certainty differences of the order of ten to twelve per cent. Differences between families in the present case were highly significant in all characters studied.

Mean squares for block differences are given in Tables I and II. Comparison with mean squares for error shows that there were significant differences between blocks in all characters in Malvi 9, and in stand and ginning percentage in Malvi 1. Arrangement in blocks has therefore improved the efficiency of the experiment very largely in Malvi 9, and quite appreciably in Malvi 1, where the blocks were much smaller. The blocks were long and narrow, and further improvement is to be expected if they are made more nearly square [Hutchinson and Panse, 1935, 1].

TABLE III

Summary of experimental details and standard errors per cent for stand and yield in replicated progeny row trials of jowar, tur, *wheat, linseed and groundnut*

——	*Jowar*	*Tur*	Wheat	Linseed	Groundnut
Experimental details—					
Number of progenies	50	40	45	23	12
Number of replications	10	6	4	5	10
Block size	58 ft. 4 in. × 10 ft.	40 ft. × 20 ft.	45 ft. × 4 ft.	26 ft. 10 in. × 2 ft. 6 in.	24 ft. × 10 ft.
Plot size	10 ft. × 1 ft. 2 in.	10 ft. × 2 ft.	4 ft. × 1 ft.	2 ft. 6 in. × 1 ft. 2 in.	10 ft. × 2 ft
Standard errors per cent—					
Stand	...	11·8	20·5	...	6·1
Crude yield	11·9	17·8	21·7	10·3	10·2
Adjusted yield	...	13·1	17·2	...	8·5
Significance of block differences—					
Stand *P*	...	Very small	Large	...	Large
Crude yield *P*	Large	Very small	Very small	Very small	0·01

In Table III are given details of replicated progeny row trials of *jowar* (*Sorghum durra*), *tur* (*Cajanus indicus*), wheat, linseed and groundnut, to illustrate the efficiency of the technique for a range of crops of different habits and seasons of growth.

In all cases each plot was a single row. With the very small plots (2 ft. 6 in. long) of linseed, a standard error for yield of ten per cent was obtained with five replications only. There was not enough seed per plant to allow of a greater replication. With *tur*, a ten-foot row and six replications gave a standard error for yield of about eighteen per cent. Clearly, it would be preferable to increase the number of replications to ten, and if necessary reduce the row length correspondingly. *Jowar* and groundnut gave yield standard errors of twelve and ten per cent with ten-foot rows and ten replications. In wheat, the amount of seed available for sowing each progeny was very limited, as only one ear per plant had been taken in the original selection. Only four replications were possible, and the standard error was correspondingly high (about twenty-two per cent). Stand was analysed in the *tur*, wheat and groundnut experiments. In all cases its standard error per cent was lower than that of crude yield. Adjustment of yield for variation in stand reduced the yield standard errors considerably.

Arrangement of the experiments in blocks increased the efficiency of the yield comparisons in all crops except *jowar*, large and highly significant portions of the variance being removed. Of the three crops on which stand was studied only in *tur* was there a significant block effect. Blocks were in all cases long and narrow (Table III). As has been pointed out above their efficiency would be improved by arranging the plots in such a way as to make the blocks approximately square.

That the accuracy of the experiments is such as to provide the plant breeder with valuable information is shown by the fact that significant differences between progenies were detected in all experiments save that on linseed.

(*b*) *Compact family blocks*

When a randomised block trial includes groups of progenies from different families, a separate variance can be calculated for each family. Each within family variance is divisible into two parts, that between progenies and that within progenies (error). The significance of the first part can be tested against the second in the ordinary way. Below are given frequency arrays of mean adjusted yield (in grms. per plot) of progenies of the five families of Malvi 9 in the 1934-35 experiment discussed above (Table I).

Frequency arrays of mean adjusted yield in grms. per plot

Family.	24	26	28	30	32	34	36	38	40	42	44	46	48	50	52	54	56	58	60	62	64	66	68	70
13	1	...	...	3	...	...	1	2	1	1	...	...	...	...	...	...	...	...	...	...	...	1	...	...
15	...	...	...	...	...	1	2	2	2	2	1	...	...	...	...	...	...	...	...	...	...	...	...	...
17	...	...	...	...	...	...	2	...	1	1	...	1	1	1	1	1	...	...	...	...	1	...	...	...
19	...	...	...	...	...	...	...	...	...	...	1	1	...	...	2	2	3	...	...	...	...	...	...	1
20	...	...	...	...	...	...	...	...	1	...	...	1	1	...	2	2	1	...	1	1	...	...	...	...
Total .	1	...	...	3	...	1	5	4	5	4	2	3	2	1	5	5	4	...	1	1	1	1	...	1

In all families except 15 there were large differences between progenies in yield, and as might have been expected the analyses of variance for the individual families showed that the differences were significant in families 13, 17, 19 and 20. The total, however, forms a much less heterogeneous group than its components, and if allowance is made for differences in family means, the distribution becomes quite compact. In agreement with this, in the main analysis the mean square due to differences between progenies within families was no greater than that due to error (Table 1, adjusted yield), and the misleading conclusion was reached that differences between progenies were entirely accounted for by differences between family means. Such results are to be expected in plant breeding material, where the distribution of the variance between families will frequently be unequal. It is therefore desirable to conduct the analysis in two stages, first examining each family separately for the significance of the differences between its progenies, and secondly examining the significance of differences between families.

This alteration in the method of analysis made possible a considerable modification in the experimental layout. Since it was intended only to compare progenies in the same family, it was possible to randomise the progenies of each family in a compact family plot. The family plots were then randomised together in the main block.

In 1935-36 a ' compact family block ' experiment was laid out with Malvi 9 cotton. Six families were included, and there were therefore six main plots per block. Five progenies were taken in each family, so that each family plot was composed of five randomised progeny rows. Each progeny row consisted of five plants spaced one ft. apart, and rows were two ft. apart. Each main plot, therefore, was 5 ft. × 10 ft. There were ten replications. A diagram of the layout is given in Fig. 3. The analysis of variance was made in two stages. First from the main plot values the variance due to family differences was analysed. Then, from the sub-plot values the variance due to progeny differences was analysed in each family separately. In Table IV are given as an illustration, summary analyses of variance of crude yield for families, and for progenies in each family.

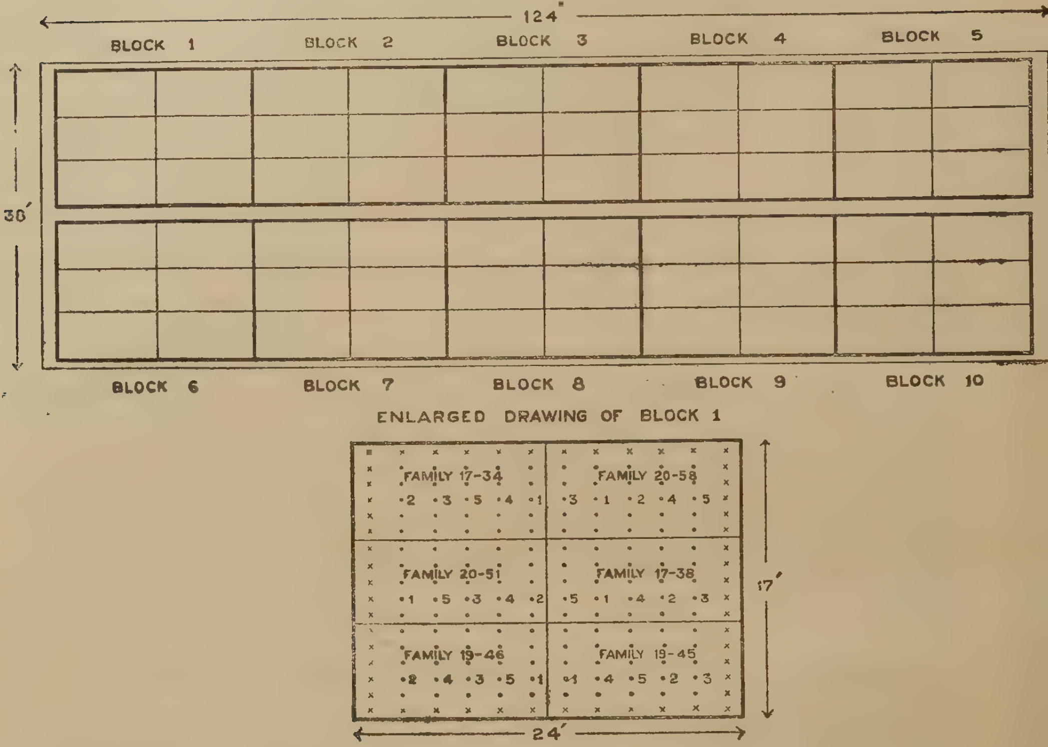

124"
BLOCK 1
BLOCK 2
BLOCK 3
BLOCK 4
BLOCK 5
38'
BLOCK 6
BLOCK 7
BLOCK 8
BLOCK 9
BLOCK 10
ENLARGED DRAWING OF BLOCK 1
FAMILY 17-34
•2 •3 •5 •4 •1
FAMILY 20-58
•3 •1 •2 •4 •5
FAMILY 20-51
•1 •5 •3 •4 •2
FAMILY 17-38
•5 •1 •4 •2 •3
FAMILY 19-46
•2 •4 •3 •5 •1
FAMILY 19-45
•1 •4 •5 •2 •3
17'
24'

FIG. 3. Field plan of compact family block trial (Malvi 9, 1935)

Experimental plots ......

Guard rows ××××

TABLE IV

Analyses of variance of crude yield (Malvi 9, 1935-36)

Due to	Between families		Between progenies within families						
				Mean squares					
				Family numbers					
	Degrees of freedom	Mean square	Degrees of freedom	17-34	17-38	19-45	19-46	20-51	20-58
Blocks . . .	9	30823	9	1275	2150	2778	2908	3168	6413
Families . . .	5	44508	Progenies 4	499	4350	5092	1415	782	921
Error . . .	45	12527	36	1011	1039	1541	1016	1501	985

Error mean squares and standard errors per cent for six characters are given for families and progenies separately in Table V.

TABLE V

Error mean squares and standard errors per cent for family and progeny comparisons in Malvi 9, compact family block arrangement, 1935-36

Character	Statistic	Progenies within families						Between families
		Family numbers						
		17-34	17-38	19-45	19-46	20-51	20-58	
Stand	Mean square.	1·15	1·15	1·15	1·30	1·22	1·21	14·1
	S. E. per cent	17·8	10·7	12·1	12·7	11·4	12·7	8·6
Yield (crude)	Mean square.	1010·5	1038·8	1541·1	1015·8	1500·8	984·6	12527·1
	S. E. per cent	25·9	14·9	17·0	15·8	16·0	14·8	10·9
Yield (adjusted)	Mean square.	528·3	804·0	529·7	421·4	674·4	551·7	5189.3
	S. E. per cent	18·7	13·1	10·0	10·1	10·7	11·1	7·1
Staple length	Mean square.	0·99	0·80	0·49	0·83	0·62	0·43	0·42
	S. E. per cent	1·2	1·1	0·8	1·1	0·9	0·8	0·5
Ginning per cent	Mean square.	7·88	6·32	3·73	5·36	9·07	6·29	2·57
	S. E. per cent	2·6	2·2	1·8	2·1	2·7	2·3	1.5

The standard errors per cent were very similar to those obtained in similar fully replicated trials, and it does not appear that the accuracy of comparison has been much affected by this new arrangement.

The compact arrangement presents a number of advantages. Comparisons between families are based on larger plots. Against this must be set the corresponding loss in replication, but judging by the magnitude of the standard errors the two probably cancel each other fairly well. Comparisons between progenies in the same family gain from the more compact arrangement of the progenies to be compared. In plant breeding work, progenies to be compared are usually closely related, and with such material it has been shown [Hutchinson and Panse, 1935, 2] that margin effect is negligible and may safely be ignored. It is sometimes desirable, however, to compare groups of progenies of markedly different habit. In such cases, margin effects may be considerable, and the compact arrangement enables each family plot to be surrounded by a row sown with bulk seed of the same family. Then between families margin effects will be eliminated by discarding the bulk row, while within families relationship will be so close that margins will be unnecessary.

The most important advantage of the compact arrangement of progenies in family plots is that the environmental contributions to the within family error variances are minimised. This, together with the random arrangement of the family plots, makes the comparison of the within family error variances a particularly useful method of comparing the relative magnitude of the genetic variance within the progenies of different families. No estimate is possible of the magnitude of the variance to be ascribed to environmental causes, but with such an experimental design it will be distributed at random between families, and significant differences in error variance may be assumed to be due to differences in the genetic component only.

Stevens has given a method of testing a set of variances for heterogeneity (Appendix to a paper by Fabergé [1936]).

Let $s_1{}^2 \ldots\ldots s_r{}^2\ s_k{}^2$ be the set of k variances which it is desired to test for heterogeneity, each with $n_1 - 1 \ldots n_r - 1\ \ n_k - 1$ degrees of freedom. Also let

$$s^2 = \frac{\sum\limits_{r=1}^{k} \left\{ (n_r - 1)\ s_r{}^2 \right\}}{\sum\limits_{r=1}^{k} (n_r - 1)}$$

Then Stevens has shown that

$$\frac{1}{2s^4} \sum_{r=1}^{k} \left\{ (n_r - 1)\ (s_r{}^2 - s^2)^2 \right\}$$

is distributed approximately as χ^2 with $k-1$ degrees of freedom.

In Table VI are given error sums of squares and degrees of freedom corresponding to the variances given in Table V for five important characters in the families of Malvi 9 included in the 1935-36 experiment.

TABLE VI

Error sums of squares and degrees of freedom in six families of Malvi 9, 1935-36

Character	Statistic	17-34	17-38	19-45	19-46	20-51	20-58	Total
Stand . .	Sum of squares . .	41·60	41·48	39·12	46·72	44·08	43·50	256·50
	Degrees of freedom .	36	36	34	36	36	36	214
Crude yield .	Sum of squares . .	36378	37397	52398	36568	54030	35446	252217
	Degrees of freedom .	36	36	34	36	36	36	214
Adjusted yield .	Sum of squares . .	18492	28141	17479	14749	23606	19310	121779
	Degrees of freedom .	35	35	33	35	35	35	208
Staple-length .	Sum of squares . .	27·64	28·73	15·29	28·25	21·21	13·90	135·02
	Degrees of freedom .	28	36	31	34	34	32	195
Ginning per cent	Sum of squares . .	228·6	228·0	115·7	182·3	308·5	211·4	1274·4
	Degrees of freedom .	29	36	31	34	34	32	196

For each character s^2 is determined by dividing the total in the sum of squares line by the total in the degrees of freedom line in Table VI. Then, s^2 is subtracted in turn from each family mean square (given in Table V) and the difference squared and multiplied by its own degrees of freedom. The sum of these products divided by $2\,s^4$ is the value of χ^2 (with five degrees of freedom) for heterogeneity in variance. Below are given the values of χ^2 for each character with corresponding values of P.

Character	χ^2	P
Stand	0·223	$>$0·99
Crude yield	4·125	0·5
Adjusted yield	4·590	0·5
Staple-length	7·509	0·2
Ginning per cent	6·679	0·2

The unexpectedly high value of P for stand is no doubt due to the effect of the method of sowing and subsequent thinning in increasing uniformity. There is no heterogeneity in variance in any of these characters. As the Malvi 9 strain had been self-fertilised for eight or nine generations, heterogeneity in variance was not likely to occur.

Other tests are also found useful for the study of group variances based upon criteria given by Neyman and Pearson in their paper "On the problem of *k* samples" (*Bulletin de l'Academie Polanaise des Sciences et des Lettres, A*, 1931); and these have been outlined in *The Journal of the Royal Statistical Society*, Part I, 1935.

(c) *Partial analysis*

The labour of computation involved in the analysis of a large replicated progeny row experiment is very considerable, and even though it is amply repaid by increased efficiency in breeding work, there is a very definite limit to the amount that can be done by a limited staff.

The amount of computation can be much reduced by judicious selection on family comparisons before progeny analyses are undertaken. In 1935 a compact family block experiment was laid out with eighteen families of *durum* wheat. Each family consisted of five progenies, and the experiment was laid out in six randomised blocks. Each progeny plot consisted of a single row 7 ft. 6 in. long. Fifteen seeds were sown in each plot. Rows were one ft. apart, so that each family plot was 7 ft. 6 in. × 5 ft., and contained five rows. A summary of the analyses of variance of family differences in stand, yield, and yield adjusted for stand is given in Table VII, and in Table VIII are given family means. Families are arranged in order of merit for each character, and families which do not differ significantly are bracketed together.

TABLE VII

Summary of analyses of yield and stand differences in families of durum *wheat*

——	Degrees of freedom	Mean square		
		Stand	Yield (crude)	Yield (adjusted)
Families	17	47·7	44239	27231
Error	85	28·7	16817	7106*
Standard error per cent . .	..	13·9	14·2	9·3
P	..	0·05	0·01	0·01

*Degrees of freedom reduced by one for adjustment.

Table VIII

Order of merit and significance of differences in families of durum *wheat*

Stand		Yield (crude)		Yield (adjusted)	
Family	Mean	Family	Mean	Family	Mean
Dhar	21·0	B 14	573	B 14	548
E 88	19·8	E 69	467	E 69	436
R 42	18·3	Dhar	422	E 111	418
K B	17·8	R 42	420	E 70	394
R 14	17·7	R 14	413	E 118	393
E 69	17·5	E 70	391	R 14	378
B 14	17·0	E 88	386	R 42	373
E 91	16·7	E 91	373	R 107	370
E 102	15·8	K B	362	K P	369
E 70	15·7	K P	360	E 95	358
K P	15·3	E 102	357	E 91	357
R 107	14·5	E 118	352	E 102	354
E 112	14·3	R 107	345	E 112	352
E 95		E 111	336	E 28	328
E 82	13·8	E 95	329	K B	324
E 118	13·7	E 112	323	Dhar	
E 111	11·5	E 82	273	E 88	319
E 28	9·5	E 28	208	E 82	311

Families enclosed in the same bracket do not differ significantly from each other.

Four families, B 14, E 69, R 42 and R 14, did well in stand and yield both crude and adjusted, and must be examined in detail for selection of material for the next generation. Three families, Dhar, E 88 and K B, did well in stand, but badly in adjusted yield. They will be worthy of further selection only if there are significant differences between progenies in adjusted yield. Three families, E 111, E 70, and E 118 gave good adjusted yields, but had poor stands, and they will be worthy of further selection only if there are significant differences between progenies in stand. The remaining eight families were mediocre to poor in both characters, and may be discarded without further analysis.

The within-family analyses which were carried out are summarised in Tables IX and X.

TABLE IX

Progeny means, standard errors per cent and significance of differences in the four best families of durum *wheat*

Family / Progeny	B 14			E 69			R 14			R 42		
	Stand	Yield (gms.)		Stand	Yield (gms.)		Stand	Yield (gms.)		Stand	Yield (gms.)	
		Crude	Adjusted		Crude	Adjusted		Crude	Adjusted		Crude	Adjusted
1 . . .	3·2	115	124	3·7	99	94	3·3	42	44	3·2	72	87
2 . . .	4·2	142	120	3·8	95	84	4·0	66	62	4·8	106	71
3	3·3	110	112	3·2	87	98	3·8	107	104	3·5	71	77
4	2·8	89	111	3·3	119	124	3·2	101	104	3·3	78	88
5	3·5	118	114	3·5	68	68	3·3	97	99	3·5	92	97
Standard error per cent	19·4	27·6	17·0	22·4	30·2	15·3	9·0	19·8	18·8	19·3	31·8	19·8
P . . .	large	large	large	large	large	large	large	0·05	0·05	large	large	large

TABLE X

Progeny means, standard errors per cent, and significance of differences in six promising families of durum *wheat*

Family / Progeny	Dhar			K B			E 88			E 70	E 111	E 18
	Stand	Yield (gms.)		Stand	Yield (gms.)		Stand	Yield (gms.)		Stand	Stand	Stand
		Crude	Adjusted		Crude	Adjusted		Crude	Adjusted			
1	4·5	88	84	3·3	64	70	3·3	66	75	3·0	1·7	2·8
2	4·2	90	91	3·3	56	62	5·2	99	81	3·0	2·7	3·2
3	3·2	70	83	2·7	78	100	3·7	58	62	4·3	2·5	2·0
4	4·7	82	76	3·7	79	76	4·7	85	74	3·2	1·8	1·7
5 . .	4·5	92	88	4·8	85	54	3·0	79	94	2·2	2·8	4·0
Standard error per cent	14·6	16·1	13·3	21·9	31·0	17·1	19·4	24·6	16·0	18·4	27·4	15·9
P .	large	large	large	large	large	0·05 (3 *vs.* rest)	large	large	large	large	large	0·05

In the four best families (Table IX) there were no significant, or even suggestive, differences in stand. In yield in R 14 progenies 1 and 2 were significantly inferior to the other three both with and without adjustment for stand. There were no other significant differences, but after adjustment for stand, the yield of progeny 4 in E 69 appears sufficiently superior to the rest to justify further trial. Of the three other families examined for adjusted yield (Table X), Dhar and E 88 showed no significant within family differences. In K B, progeny 3 was significantly superior to the rest. Of the three examined for stand only, E 70 and E 111 showed no significant differences. In E 118 progeny 5 was significantly superior to the rest.

The best plants were selected from the following progenies as parents of progeny rows in the following year:

R 14 progenies 3, 4 and 5.
E 69 progeny 4.
K B progeny 3.
E 118 progeny 5.

All seed of families B 14 and R 42, and (after single plant selection) the remaining seed of E 69 and R 14 progenies 3, 4 and 5 was bulked family-wise for trial against local in a replicated trial with 1/100 acre plots.

In all, therefore, out of eighteen families, it was only necessary to analyse seven completely, and three more for one character only. Not only was the labour of computation halved in the current season, but by making single plant selections only in families which showed significant, or highly suggestive differences between progenies, the labour of the succeeding experiment will be substantially reduced. Plants were selected from six progenies only, which will give an experiment containing six families as against eighteen. Nothing is to be gained by further single plant selection in families showing no differences between progenies, and they are accordingly bulked for multiplication and full-scale trial.

Standard errors of progeny comparisons were large, though in most cases adjustment for stand improved the accuracy of the yield comparison very considerably. The standard error per cent of the stand comparison is exaggerated by the fact that germination was very poor. There were only six replications, and this is scarcely adequate.

In many cases, material can be discarded on field examination before harvest. A further economy in labour is then possible. If the experiment is arranged in compact family blocks, all progenies of an inferior family may be harvested in bulk. The family plot bulks can be included in the main family analysis for the sake of completeness, but the labour of separate harvesting and weighing of progeny plots is saved. In a completely randomised experiment any inferior progeny may be cut out and discarded at harvest time, and only the best progenies harvested plot-wise, weighed and analysed. This device is particularly valuable where experimental facilities are poor. In 1934 a replicated progeny row trial with sesamum was conducted at Dhamnod, forty-five miles from Indore. Forty-eight progenies were laid out in a randomised block experiment, replicated ten times. At harvest it was necessary to take the material to Indore for drying and weighing. Preliminary observations were taken plot-wise on earliness, stand, and vigour. In making estimates of such characters as earliness and vigour, a randomised layout has the great advantage of avoiding bias and providing an estimate of the accuracy of judgment. As estimates from different replicates of the same progeny agreed satisfactorily, inferior progenies were discarded with confidence. At harvest, all progenies except the best ten were first cut out and removed. The remainder were then harvested plot-wise and taken to Indore for examination. The material was thereby reduced from 480 plots to 100 on preliminary observations alone, without affecting the validity of the analysis of variance of the selected progenies.

(*d*) *Efficient utilisation of available seed*

In progeny row breeding work the amount of seed available often varies widely among the plants selected as parents of the next generation. Where the minimum available is sufficient to sow ten or more plots of the size decided upon (as for instance, with *jowar*), this variation is of no consequence. In other cases, the reduction of the number of replications to that for which the lowest yielding plant provides sufficient seed, will result in a serious loss of efficiency.

We are indebted to Mr. F. Yates, Chief Statistician, Rothamsted, for suggesting the two following methods of utilising most of the available seed in such cases.

Where most of the selected parent plants have given about the same amount of seed, and two or three have given considerably less, a plot size and number of replications may be employed which will use up nearly all the seed of the higher yielding group. The seed of progenies of the lower yielding group is used to sow as many plots as the amount available permits, and the remaining plots allotted to those progenies are sown with bulk seed, either surplus from higher yielding plants, or from related strains. In calculating the results, data for the plots sown with bulk are estimated by the 'missing plot technique' [Yates, 1933].

The 1934 linseed progeny row trial was laid out with seven randomised blocks. Of these, five contained all twenty-three progenies. The sixth block contained seventeen plots, and the seventh, fifteen plots. In this case the plots of the missing progenies were omitted from the last two blocks, and the blocks were reduced in size. It appears preferable to sow the missing plots with bulk, however, in order to maintain a constant block size. A diagram of the layout with constant block size is given in Fig. 4. The estimation of the missing values would be more efficient if the bulk-sown plots were distributed at random in all blocks instead of being concentrated in the last two.

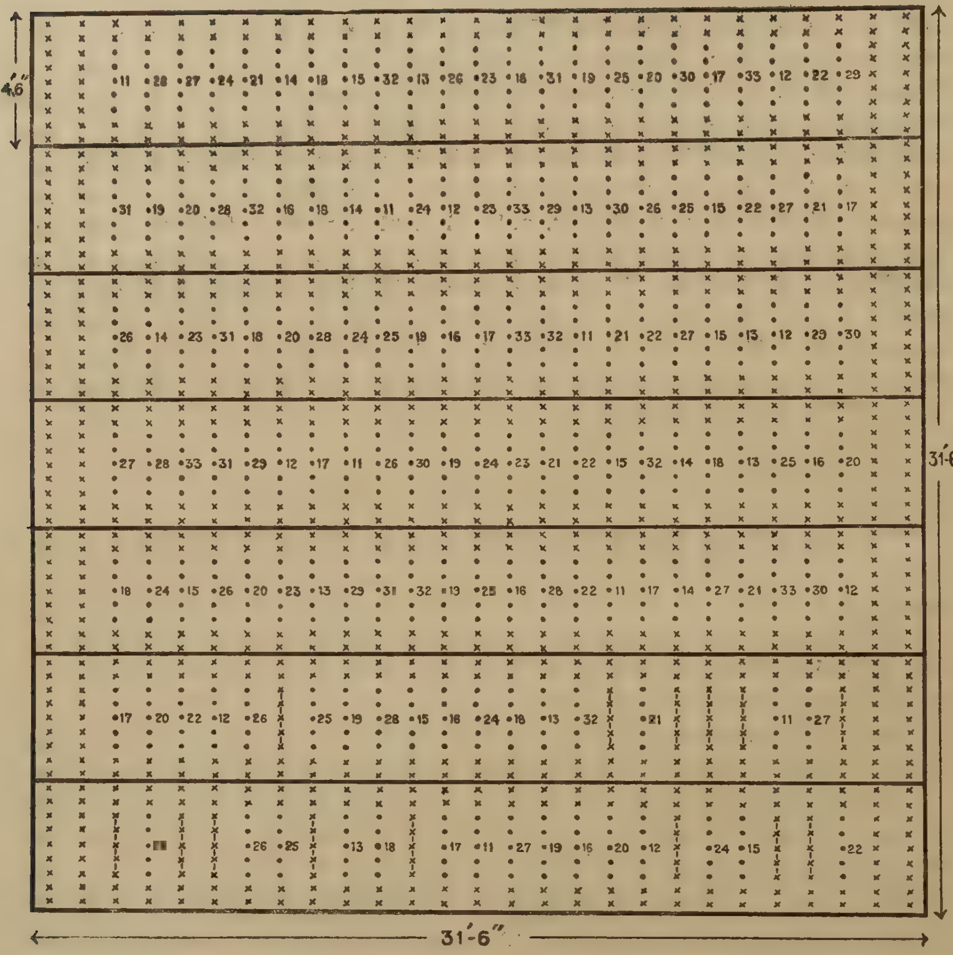

4'6"
31'6"
31'-6"
•11 •28 •27 •24 •21 •14 •18 •15 •32 •13 •26 •23 •16 •31 •19 •25 •20 •30 •17 •33 •12 •22 •29
•31 •19 •20 •28 •32 •16 •18 •14 •11 •24 •12 •23 •33 •29 •13 •30 •26 •25 •15 •22 •27 •21 •17
•26 •14 •23 •31 •18 •20 •28 •24 •25 •19 •16 •17 •33 •32 •11 •21 •22 •27 •15 •13 •12 •29 •30
•27 •28 •33 •31 •29 •12 •17 •11 •26 •30 •19 •24 •23 •21 •22 •15 •32 •14 •18 •13 •25 •16 •20
•18 •24 •15 •26 •20 •23 •13 •29 •31 •32 •13 •25 •16 •28 •22 •11 •17 •14 •27 •21 •33 •30 •12
•17 •20 •22 •12 •26 •25 •19 •28 •15 •16 •24 •18 •13 •32 •21 •11 •27
•26 •25 •13 •18 •17 •11 •27 •19 •16 •20 •12 •24 •15 •22

FIG. 4. Field plan of replicated progeny row trial (linseed, 1934).

Experimental plots . . . .
Guard rows × × × × ×
Plots sown with bulk × — × — × — ×

The five complete blocks were analysed separately for comparison. Summaries of the analyses of the five complete blocks and of all seven blocks are given in Table XI. (Some information from the five blocks has already been given in Table III.)

TABLE XI

Analyses of variance of yields of linseed progenies

Due to	Five complete blocks		Seven blocks (two incomplete)	
	Degrees of freedom	Mean square	Degrees of freedom	Mean square
Blocks	4	118·65	6	106·70
Progenies	22	10·69	22	14·10
Error	88	14·30	132—14=118	13·83
Standard error per cent—				
Mean of 5 plots	..	10·31	..	10·48
Mean of 7 plots	..	..	..	8·87

The number of degrees of freedom available for the estimation of error was reduced by fourteen (the number of missing plots) in the seven-block calculation, but there remained 132, as against eighty-eight in the five-block calculation. The standard error per cent for those progenies which appeared in all blocks was reduced from 10·31 to 8·87, a substantial gain. For those progenies which appeared in five blocks only, it remained practically unchanged.

Where the amount of seed from selected parents varies widely, it is often not possible to arrange so simple a layout. In such cases, the number of blocks should be equal to the number of plots that can be sown with the seed available from the lowest yielding parent. Then parents yielding twice as much seed can be included in two plots per block, those yielding three times as much seed, in three plots per block, and so on.

In 1935, a progeny row trial was laid out with five Upland cotton progenies, *A*, *B*, *C*, *D* and *E*. Each plot was a single row of five holes, and seed was sufficient to sow the following numbers of plots per progeny:

Progeny	Number of plots
A	16
B	11
C	16
D	16
E	4

The experiment was laid out in four blocks. *E* appeared once in each block. *A*, *C* and *D* appeared four times in each block, and *B* appeared three times in the first three blocks and twice in the fourth, the remaining plot being sown with bulk. A diagram of the layout is given in Fig. 5. Yields of *kapas* per plot are given in Table XII.

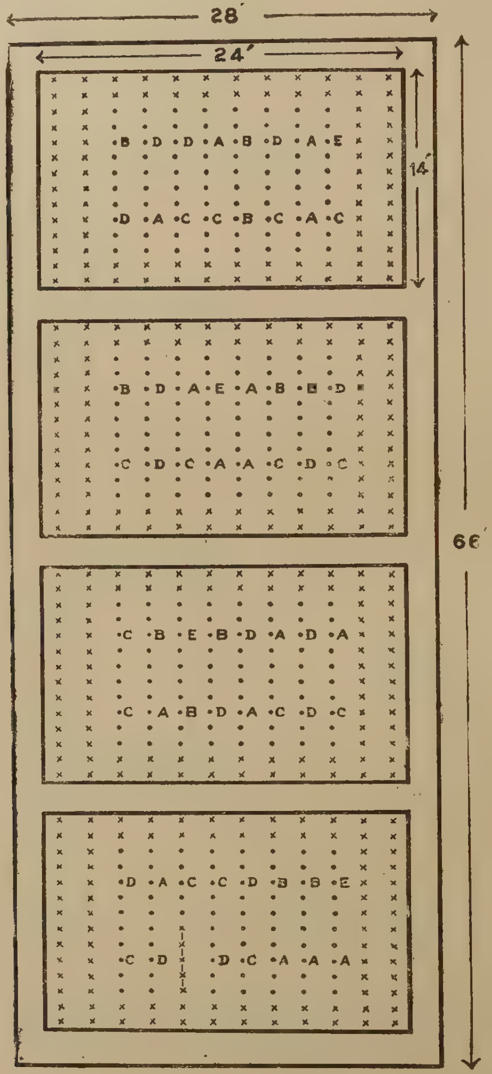

28'
24'
14'
66'
B D D A B D A E
D A C C B C A C
B D A E A B B D
C D C A A C D C
C B E B D A D A
C A B D A C D C
D A C C D B B E
C D D C A A A

FIG. 5. Field plan of replicated progeny row trial (Upland cotton, 1935).

Five progenies *A* — *E*.
Experimental plots ....
Guard rows × × × × ×
Plot sown with bulk × — × — × — ×

The estimation of the missing value for progeny *B* involves a modification of Yates' [1933] equation, and is considered in detail in the Appendix. In the present case:

The number of plots per block . . . . . . $= p^1 = 16$
The number of plots of the progeny with the missing plot . $= q^1 = 12$
The number of plots per block of the progeny with the missing plot . . . . $= a = 3$
The sum of the known yields in the progeny with the missing plot . . . . $= P = 707$
The sum of the known yields in the block with the missing plot . . . . $= Q = 727$
The sum of all known yields . . . . . . $= T = 3{,}604$

The value of the missing plot may now be estimated by substitution in equation (4) of Appendix I:

$$p^1 q^1 x - p^1 x - q^1 x + ax = p^1 P + q^1 Q - a T$$

Substituting

$$167\ x = 9224$$
$$x = 55{\cdot}2$$

The estimate of the missing value, and totals and means including it, are inserted in brackets in Table XII.

TABLE XII

Progeny rows of Upland cotton, 1935 (yields of kapas in *grms.)*

Progeny	Block 1	2	3	4	Total	Mean
A	44	68	51	33	914	57·13
	55	42	70	71		
	55	82	40	59		
	59	79	58	48		
B	108	74	75	x (55)	707 (762)	(63·50)
	45	82	53	35		
	48	62	76	49		
C	61	64	63	10	787	49·19
	54	59	53	53		
	56	76	35	41		
	51	38	44	29		
D	111	34	63	45	1043	65·19
	58	34	130	54		
	69	30	87	116		
	24	59	71	58		
E .	49	33	45	26	153	38·25
Total .	947	916	1014	727 (782)	3604 (3659)	

In the analysis of variance, the sum of squares due to progenies is

$$S\left\{\frac{(\text{progeny total})^2}{\text{number of plots per progeny}}\right\}-\frac{(\text{grand total})^2}{\text{total number of plots}}$$

As the number of plots per progeny differs in different progenies, the contribution of each progeny must be calculated separately, instead of summing the squares of the progeny totals and dividing by the common plot number, as is usually done when the number of plots per progeny is constant. Below is given the analysis of variance of crude yield of *kapas*.

——	Degrees of freedom	Sum of squares	Mean square	1/2 $\log_e$	z
Blocks	3	1,782	594·0		
Progenies	4	3,970	992·5	3·450	0·394
Error	55	24,841	451·7	3·056	

$P = 0 \cdot 05$ when $z = 0 \cdot 463$

Differences between progenies in crude yield were not significant.

The standard errors of the progeny means will vary according to the number of plots per progeny, and must therefore be calculated separately for progenies with different numbers of plots. Below are given standard errors as per cent of the grand mean, for stand, crude yield, and yield adjusted for stand. The experiment was very efficient for sixteen plot progenies, good for the eleven plot, and fair for the four plot progeny.

Progenies	Number of plots	Standard error per cent		
		Stand	Crude yield	Adjusted yield
A, *C* & *D*	16	6·9	9·3	6·0
B	11	8·3	11·2	7·2
E	4	13·8	18·6	12·0

Where the value of z shows that significant differences exist between progenies, it is necessary to calculate separate standard errors of differences for comparisons involving different plot numbers. Differences between yields adjusted for stand were highly significant. Mean adjusted yields per plot were as follows :

Strain	*A*	*B*	*C*	*D*	*E*
Yield (grms.)	56·8	60·5	51·5	67·5	36·7

The variance of a single plot was 123·4, and the variances of the progeny means :

A, C & D	123·4/16
B	123·4/11
E	123·4/4

The standard errors of the differences between progeny means were as follows :

Between progenies with 16 plots . . . $=\sqrt{\frac{123\cdot4\times2}{16}}=3\cdot93$

Between a progeny with 16 plots and *B* (11 plots) $=\sqrt{\frac{123\cdot4}{16}+\frac{123\cdot4}{11}}=4\cdot35$

Between a progeny with 16 plots and *E* (4 plots) $=\sqrt{\frac{123\cdot4}{16}+\frac{123\cdot4}{4}}=6\cdot21$

Between B (11 plots) and *E* (4 plots) . . $=\sqrt{\frac{123\cdot4}{11}+\frac{123\cdot4}{4}}=6\cdot49$

Below, the strains are arranged in order of yield and taking a difference equal to or greater than twice its standard error as significant, those which do not differ significantly are included within a bracket :

$$\begin{array}{lccccc}
\text{Strain} & D & B & A & C & E \\
\text{Yield per plot} & 67\cdot5 & 60\cdot5 & 56\cdot8 & 51\cdot5 & 36\cdot7 \\
 & \multicolumn{2}{c}{\underbrace{\hphantom{67\cdot5 \quad 60\cdot5}}} & & & \\
 & & \multicolumn{2}{c}{\underbrace{\hphantom{60\cdot5 \quad 56\cdot8}}} & & \\
 & & & \multicolumn{2}{c}{\underbrace{\hphantom{56\cdot8 \quad 51\cdot5}}} &
\end{array}$$

(*e*) *Small bulk trials*

Testing of plant breeding material against the locally cultivated type is desirable at the earliest possible stage. Only on the results of such tests can the breeder satisfy himself that his material is of sufficient promise to justify the expenditure of his efforts. The inclusion of control plots of the local type in progeny row trials is undesirable. Local mixtures will usually be very much more heterogeneous than single plant progenies, and small plot samples may not be properly representative of the type. Their inclusion may also result in a more irregular distribution of the error variance, and an unnecessary increase in its magnitude.

At the end of a season's trial, the best progenies are selected from a progeny row trial for further test. From them the best plants are selected as parents of the progenies of the next generation. The seed of the remaining plants may be bulked for test against the local type in a larger scale experiment. To these may be added bulks of progenies which did well but which were not judged of sufficient merit to be selected for continuation in the progeny row trial.

An example may be given from the 1935 breeding trials with *jowar*. In the 1934 progeny row trial, in order to facilitate single plant selection, yield of heads was studied before threshing and corrections for threshing percentage applied subsequently. For simplicity, only data for crude yield of heads are given. Threshing percentage averaged about seventy-two.

The ten best progenies did not differ significantly from each other, and yielded (in grms. of heads per plot) as follows :

Progeny	Yield	Progeny	Yield
20	187	26	160
3	180	44	158
11	178	18	158
5	176	17	157
12	162	1	156

Of these, progenies 3, 11, 12, 18, and 20 were selected for their superior grain quality for inclusion in the 1935 progeny row trial. The ten best heads were selected in each progeny as parents of the 1935 progeny rows. The 1935 progeny row trial, therefore, contained fifty progenies, ten in each of five families. It was laid out in ten completely randomised blocks. Below are given yields of heads (in grms. per plot) of the five families. Families not differing significantly in yield are included in a bracket.

Family .	18	12	11	20	3	St. error (per cent)
Yield . .	138	133	133	125	117	3·7

Ten family bulks were made up from the heads not selected for the replicated progeny row trial. These were laid out with the local as control in six randomised blocks, with 1/50 acre plots, and one row at each side and four ft. at each end of each plot were allowed for non-experimental margins. Studies of margin effect [Hutchinson and Panse, 1935, 2] led to the conclusion that with trials of closely related strains, such as are likely to emerge from plant-breeding experiments, side margins are unnecessary, and they were omitted from later small bulk trials. Below are given yields of heads (in lbs. per acre) from the bulk trial. Families whose yields do not differ significantly are bracketted together.

Family . .	26	5	17	20	12	44	11	18	Local	1	3
Yield .	2138	2130	1941	1720	1712	1698	1577	1480	1440	1424	1317

Standard error per cent = 4·7

The results of the two trials differ markedly with regard to the relative value of families 18 and 20. In the bulk trial 20 out-yielded 18 by a significant amount, whereas in the progeny row trial 18 out-yielded 20 considerably, though not significantly. As there were significant differences between progenies in all families, this discrepancy may safely be ascribed to the chance selection of better parents for the progenies of 18 than of 20. Both trials show family 3 to be inferior, and it may be discarded with advantage.

Selection among the ten best 1934 progenies for grain quality resulted in the elimination from the progeny row test of the whole of the highest yielding group of strains. Their continuation in bulk made it possible to recover them for the 1936 progeny row trial by selection of the best heads from the bulk plots, and it is a very valuable function of such bulks to serve as a safeguard against accidental discarding of superior material.

In spite of the variability of the material (as shown by the existence of significant differences between progenies), the bulk trial gave a low error and a good differentiation between strains. Seven out of the ten tested were significantly superior to the local control—a very satisfactory demonstration of the value of the material—and only one was significantly inferior.

The value of small bulk trials at an early stage was well shown in a selection trial with *tur* (*Cajanus indicus*). In the second year of selection, a small bulk trial and a compact family block progeny row test were carried out in parallel. From the small bulk trial data, it appeared that a number of strains were about equal to the local in yield, but none were significantly superior. The material as a whole, therefore, was not of any great value. The existence of significant differences between progenies of families about equal in yield to the local demonstrated the possibility of improvement by selection, and the best of such progenies were therefore retained, and the whole of the rest was discarded. In the absence of early small bulk trials, many comparatively unpromising strains might have been carried on for several seasons.

III. Discussion

The present paper is confined to consideration of the technique of replicated progeny row trials. The valuable results already obtained by its use will be reported in later papers in this series.

In plant breeding, the influence of recent development in statistical methods has hardly been felt beyond the sphere of variety trials. The limited and variable amounts of seed available, the large numbers of progenies to be tested, and doubt as to the validity of comparisons of heterozygous material, have all contributed to reluctance to adopt methods developed for large plots and abundant supplies of seed. Usually nothing more has been attempted than the inclusion of control plots at regular intervals. Some workers employ two or three replications systematically arranged, and in Egypt systematic replication has been carried further, and the 'Purity Chequer' developed [Brown, 1932]. Trought [1934] has described a method of testing small family bulks which involves sowing a 100-metre family row with a 100-metre control row on either side of it, and guard rows outside these. Comparisons are made in sets of three plants, one family plant against the mean of two control plants which correspond exactly to it in position and spacing.

It has been shown elsewhere [Hutchinson and Kubersingh, 1936] that with the simpler arrangements a large and important part of the genetic variability available escapes detection, and the methods described in the present paper have been devised to reduce this loss. That they actually do so is abundantly proven by the detection of considerable and significant differences between progenies of inbred and by current standards 'pure' strains of cotton.

The essential difference between the replicated progeny row technique here advocated, and the 'Purity Chequer' of Brown and the 'Line test' of Trought lies in the use of randomisation in the former. The disadvantages of systematic arrangements have been discussed in detail by Fisher [1935], who concludes, "The failure of systematic arrangements came from not recognising that the function of an experiment was not only to make an unbiassed comparison, but to supply at the same time a valid test of its significance." The 'Purity Chequer' provides no valid estimate of error, while Trought's method gives neither an unbiassed comparison nor an estimate of error. The special precautions necessary in sowing, the high level of cultivation and fertility considered necessary for the 'Purity Chequer', and the extreme accuracy in spacing essential for the 'Line test' are all disadvantages to which the replicated progeny row technique is much less subject. Trought considers that the observation that root competition sometimes increases the difference between strain and control by giving an extra advantage to the type with the best root system, is an advantage rather than a defect in his method. There is no assurance that a strain which gains by the competitive advantage of its root system will be superior to its rival in large plots where there are no inferior root systems to be crowded out. Studies of margin effect in variety trials [Hutchinson and Panse, 1935,2] have shown that there is no predictable relationship between the ratio of the yields of the central plots of two varieties and of their border rows. Trought's argument is therefore invalid, since his control will be disturbed in varying and unknown directions. In addition the fundamental criticism, that a biassed result is of little value, stands even if the bias is in favour of the better strain. Trought states that random arrangement of thirty or more families would be quite impossible on account of the area they would cover. Since, in the 'Line test', four-fifths of the area (two control rows and two guard rows per family tested) is covered by control material, a randomised block arrangement would actually result in a very large saving in space, besides providing direct comparisons between all families in the trial. If the number of families to be included is too great to give an efficient size and shape of block, Yates' [1936] pseudo-factorial arrangement can be adopted. It may be suggested that if the replicated progeny row technique were used, the number of strains to be tested in small bulks would be considerably reduced, and would be no more than would be conveniently manageable in small bulk randomised block trials.

With the 'Purity Chequer' no attempt is made to estimate yield, and with neither the 'Purity Chequer' nor the 'Line test' is it possible to study the differences between strains in germinating capacity and ability to establish a satisfactory stand under approximately normal cultivation conditions. Germination and stand are of great importance, and with the replicated progeny row technique it is possible to study both with accuracy, and still to obtain a satisfactory estimate of yield from which the effect of differences in stand can be removed by the analysis of covariance.

Eye judgments of such characters as vigour, lodging , or average rust infestation, can be interpreted with confidence when based on plot-to-plot examinations in randomised progeny row trials. Eye estimates are always liable to bias from the natural tendency to judge by the contrast between neighbouring plots. With systematic arrangements this may be serious, whereas with random arrangements, mean values will be unbiassed. Further, if the classification is done on a series of numerical grades, a standard error can be calculated from a randomised experiment, and the reliability of the classification directly tested.

With increased accuracy of comparison there arises the possibility of studying the differential response of progenies or families of the same strain in different years. It is well known that one of the great risks involved in the distribution of pure strains is that they are of necessity tested over only a limited range of seasons, and they may fail to maintain their promise when they encounter greater environmental differences. Controlled testing from the earliest stages, both in progeny rows and small bulks, adds greatly to the knowledge of response to seasonal variations which will be available when the selected strains reach the distribution stage. The material can be subjected to a steady sifting process since, though it is not possible to say that a progeny which does well in one season will meet satisfactorily the range of seasons occurring, it is always possible to discard on a single season's failure on the ground that that strain, however well it may behave on the average, does not possess the necessary flexibility of response.

The breeding methods here reported were designed as much to test the capacity of plant-breeding material to respond to further selection as to evaluate the effects of selection in previous years. Response to selection depends upon the existence of genetic variance, and the problems involved in the estimation of genetic variance will be briefly discussed.

It has been shown elsewhere [Hutchinson and Kubersingh, 1936] that in the selection work on Malvi cottons, a large part of the superiority obtained over the local type was due to the initial superiority of the foundation plants from which the improved strains were descended. The selection process within strains was very inefficient in sifting out and retaining the superior genotypes arising by segregation in the initially heterozygous material. If all that could be hoped for from selection work were the isolation of the initially superior components of a crop mixture, elaborate progeny row experiments would be unnecessary, and it would be cheaper and simpler to bulk up immediately the progenies of superior field selections, and test them in large scale plots. It is the aim of the plant breeder, however, to obtain a progressive improvement in his material by the isolation of superior types arising by segregation in the progeny of initially heterozygous selections. To do this efficiently he must be in a position to obtain answers to the following questions :

(1) Which of his original types contain sufficient genetic variance to hold out the promise of response to selection ?

(2) Which of the subsequent families are uniform, or nearly so, and therefore useless for further selection, and which among those with high means have a high genetic variance, and therefore combine an improved average performance with the promise of further response to selection in the same direction ?

The chief difficulty in the estimation of genetic variance lies in the impossibility of separating it from environmental variance. It is therefore necessary to adopt the fundamental principle of experimental design, that the incidence of factors which cannot be controlled must be equalised, or distributed at random throughout the experiment. In randomised block experiments the main environmental effects are equalised between blocks, and the within block effects are distributed at random. The design of a randomised block trial, however, does not admit of the estimation of a separate between plot variance for each progeny. Within progeny variances can be estimated from the individual plant values, and the possibilities of such estimation, after correcting the single plant values for the ratio of the mean of the block to the grand mean (to eliminate block effects) are worthy of detailed study. When groups of progenies from several families are included in a single experiment, the within progeny variance can be calculated for each family separately. Differences between the mean within progeny variances in different families may be ascribed to differences in average genetic variance, since major environmental differences have been eliminated with the block effect, and minor ones will be distributed at random. The arrangement of the progenies in compact family plots further reduces the contribution of environment to the within progeny variance, and therefore increases the accuracy of comparison. An example of the comparison of such family variances is given above, but as might have been expected with highly inbred material, no differences existed in average family variance. Of families with high means and high variances selection may profitably be continued in the best progenies. Of families with high means and low variances, the best progenies may be reserved for bulking up and testing on a larger scale, while the rest of the material may be discarded. It is important to note the division of the best material into two lots, that in which further selection is likely to be profitable, and that which has reached the limit, and may be passed on from the breeding to the testing plots to be either distributed, or discarded if it fails to reach the required standard.

A problem which arises when an attempt is made to improve the efficiency of selection methods, is that of deciding at what stage further selection in the material under test becomes unprofitable, and new types should be brought under study. The problem is, in fact, that of deciding when the genetic variance of the material is exhausted, and the comparison of average genetic variances in compact family block experiments will go a long way in assisting the breeder to decide how long to carry on selection.

The refinement of breeding technique has resulted in the detection of variability in strains hitherto considered to be ' pure ', and necessitates a reconsideration of the standard of purity required before a new type may safely be distributed. It is desirable to distinguish between commercial purity and genetic purity. Commercial purity involves no more than uniformity in agricultural response and commercial quality, and may easily be attained by material which would be considered highly heterogeneous by the breeder. Plant-breeding material will usually attain the standards required by the farmer and the trade long before it reaches the distribution stage. The breeder requires genetic purity in order to ensure the stability of the material he distributes. Agricultural conditions are such that crop varieties are constantly subject to deterioration by contamination from extraneous sources (in threshing machines and ginneries as well as by cross pollination) and it is always necessary to provide for the renewal of seed stocks from breeding centres. The standard of purity the breeder should set himself before commencing distribution, therefore, should be high enough to ensure that deterioration by segregation due to heterozygosity will be small relative to deterioration due to seed mixing and natural cross pollination. Beyond that he need not go.

When a strain has been bred and distributed and the genetic variance has dropped so low that the breeder has turned his attention to other material, there still remains the problem of the maintenance of the nucleus stock. The method commonly employed is to select in each year a number of typical, vigorous, productive plants from that year's nucleus to provide seed for the nucleus of the following year. The disadvantages of this practice are twofold. First, there is no satisfactory test of yield, and therefore no means of excluding low yielding types arising either by segregation from the residual genetic variance or by mutation. Secondly, there will always be the risk of ruining the nucleus by the inclusion in the parental group of one or more plants which owe their vigour and productivity to heterosis resulting from natural crossing. The maintenance of a small replicated progeny row trial in each year will overcome these defects. The bulk of the labour involved in such trials is labour in computation, and where very pure material is under trial, it will usually be obvious from an examination of the progeny totals that no significant differences are present. Calculations need then only be carried out on data for such characters as show differences of importance. The occurrence of progenies markedly below the mean in yield will be detected rapidly. As the standard errors of yield comparisons are rather high, it may take two years to prove conclusively the significance of a difference of sufficient importance to impair the value of the strain seriously. In such work a table of z for $P = 0 \cdot 1$ would be of great value, as the plant-breeder will be ready to discard a strain if he knows that the odds are ten to one that it is lower yielding than the standard.

The replicated progeny row technique, like other statistical methods, is a tool, and should be used with discretion. It involves care and forethought in the design of the experiment and considerable labour in computation when the data have been collected, and it is a waste of effort to use it where simpler methods will serve the same purpose. Where it is safe to make the first year's selections on eye judgments or simple measurements on single progeny rows, greater efficiency will be achieved by examining the largest possible range of material than by collecting accurate statistics on a limited number of progenies. As soon as it becomes necessary to select on characters such as yield which are greatly influenced by environmental conditions, the replicated technique becomes valuable. As the material becomes more and more homogeneous and the breeder is faced with the problem of choosing between closely related strains of similar performance, differences in characters only slightly affected by environmental changes will be so small as to be detectable only in replicated experiments. Experience has shown that the extra labour involved in the design and conduct of replicated progeny row trials is not excessive. At Indore, where it is vital for the success of the ***kharif*** (monsoon) crops to complete the sowing at the earliest possible moment after the break of the rains, no extra delay has resulted from the adoption of these methods. Speed and success at sowing time depend very largely on forethought and careful preparation of field plans and arrangement of seed so that field work is reduced to following a carefully organised programme.

IV. SUMMARY

The use of replication and randomisation in progeny row breeding work is described and illustrated from experiments on a range of agricultural crops. The standard randomised block technique was adapted to progeny row work by reducing the plot size to a single row 5 ft. or 10 ft. long according to the amount of seed available. Fluctuations in stand are inevitably large in such trials, and their effect on the estimation of yield is eliminated by the use of the analysis of covariance. Examples are given of the standard errors obtained in work with a number of crops of different habits and seasons of growth, and it is shown that with ten replications, standard errors of ten per cent of the mean, or less, may be expected for yield, and for characters such as staple-length and ginning percentage in cotton, they may be as low as two or three per cent.

For testing groups of progenies belonging to related families, the compact family block design is described involving a further restriction on the randomisation. By this method a more accurate comparison is possible between progenies of the same family, while the loss in replication for family comparisons is fairly well compensated by the increase in the size of the family plots. It is shown that the compact family block design can be used to provide a comparison between families of the average genetic variance within their progenies. The importance of this comparison for the guidance of the plant-breeder in making further selections is emphasised.

Examples are given illustrating methods of reducing the labour of computation by ignoring comparisons which are unlikely to provide information of value.

Methods suggested by Yates for overcoming the difficulties of very unequal amounts of seed from different parent plants, are illustrated by practical examples, and the ' missing plot technique ' is modified for use with one of them.

The use of small bulk trials to supplement progeny row trials in breeding work is illustrated, and their value for retaining a wider range of types than can be maintained in progeny rows is demonstrated.

Current methods and recent refinements in progeny row breeding are discussed and compared with the replicated progeny row technique. It is concluded that the latter is more efficient, more flexible, and of much wider application, than any other method. In addition to providing estimates of mean values and tests of their significance for all measurable characters, it provides a basis for the comparison of genetic variances which has given valuable information and is capable of wide extension.

V. Acknowledgments

In the development of the methods here described we have received constant advice and criticism from Mr. Kubersingh, to whose knowledge of the Indore material and understanding of the breeder's art we are deeply indebted.

To Mr. F. Yates, Chief Statistician, Rothamsted Experimental Station, we are obliged for suggesting the methods developed for the efficient utilisation of variable amounts of seed.

We have drawn freely on experimental data collected and analysed by other members of the Institute staff, and we wish to record our thanks to Messrs. Kubersingh, Ghose, Simlote, Apte, Ansari, Khargonekar, Singh, and Rajanna.

The groundnut data were collected by the Department of Agriculture, Jaipur State, to whom our thanks are also due.

REFERENCES

Brown, C. H. (1932). *Emp. Cot. Grow. Rev.* **9**, 119.
Fabergé, A. C. (1936). *Jour. Genet.* **33**, 398.
Fisher, R. A. (1935). ' Design of Experiments.' Oliver and Boyd, London.
Hutchinson, J. B. and Kubersingh (1936). *Ind. Jour. Agric. Sci.* **6**, 672.
Hutchinson, J. B. and Panse, V. G. (1935,1). *Ind. Jour. Agric. Sci.* **5**, 523.
—————————————— (1935,2). *Ind. Jour. Agric. Sci.* **5**, 671.
Irwin, J. O. (1935). *Jour. Roy. Stat. Soc.* **98**, 94-98.
Neymen, J. and Pearson, E. S. (1931). *Bull. de l' Acad. Pol. des Sciences et des Lettres* **A**, 960-81.
Trought, T. (1934). *Report and Summary of Proc. E. C. G. C. Second Conference on Cotton Growing Problems*, p. 51.
Yates, F. (1933). *Emp. Jour. Expt. Agric.* **1**, 129.
————(1936). *Jour. Agric. Sci.* **26**, 424.

APPENDIX

The estimation of a missing plot value in progenies with more than one plot per block

The missing plot value may be estimated by an extension of Yates' [1933] method of minimising the error variance. The classification is two-fold, into progenies and into blocks.

Let :

the number of progenies . . . . $=p$
the number of blocks . . . . $=q$
the number of plots per block . . . . $=p^1$
the number of plots of the progeny with the missing plot . . $=q^1$
the total number of plots . . . . $=n$

Also let :

the sum of the known yields in the progeny with the missing plot $=P$
the sum of the known yields in the block containing the missing plot . . . . $=Q$
the sum of all known yields . . . . $=T$

Then if x is the value of the missing plot, the terms in the sum of squares containing x will be :

due to progenies $\frac{1}{q^1}(P+x)^2 - \frac{1}{n}(T+x)^2$

due to blocks $\frac{1}{p^1}(Q+x)^2 - \frac{1}{n}(T+x)^2$

total $x^2 - \frac{1}{n}(T+x)^2$

and the contribution of terms containing x to the error sum of squares will be :

$$x^2 - \frac{1}{q^1}(P+x)^2 - \frac{1}{p^1}(Q+x)^2 + \frac{1}{n}(T+x)^2 \ldots\ldots\ldots (1)$$

Differentiating and equating to 0 we get :

$$p^1q^1nx - p^1n\,(P+x) - q^1n\,(Q+x) + p^1q^1\,(T+x) = 0 \ldots (2)$$

As $\frac{p^1q^1}{n}$ $(=a)$ will be the number of plots per block of the progeny containing the missing plot, equation (2) may be rewritten :

$$p^1q^1x - p^1\,(P+x) - q^1\,(Q+x) + a\,(T+x) = 0 \ldots\ldots (3)$$

$$\text{or } p^1q^1x - p^1x - q^1x + ax = p^1P + q^1Q - a\,T \ldots\ldots\ldots\ldots (4)$$

When $p^1 = p$ and $q^1 = q$, equation (4) becomes Yates' general equation for the estimation of a missing plot in a randomised block experiment.

Where there are missing plots in more than one progeny, it should be remembered that q^1 and a will have different values if the number of plots per progeny differs.

DISEASES OF THE TEA BUSH IN THE KANGRA VALLEY, PUNJAB, I

BY

H. CHAUDHURI

V. S. KAPUR

K. L. BHATIA

AND

J. S. ANAND

Department of Botany, Punjab University, Lahore

(Received for publication on 16th March 1937)

(With Plates XLIX and L)

CONTENTS

		PAGE.
I.	INTRODUCTION	565
II.	GREY BLIGHT (*Pestalozzia theae* SAWADA)	566
III.	BROWN SPOT DISEASE (*Phoma theicola* PETCH)	568
IV.	BROWN BLIGHT (*Colletotrichum camelliae* MASSEE)	569
V.	SCABBING OF LEAVES (COMBINED EFFECT OF *Phoma, Pestalozzia* AND *Colletotrichum*)	570
VI.	COPPER BLIGHT (*Guignardia camelliae* (CKE.) BUTLER)	570
VII.	INTERNAL ROOT DISEASE (*Botryodiplodia theobromae* PAT.)	571
VIII.	A STEM DISEASE (*Hendersonia theicola* CKE.)	572
IX.	RED RUST OF TEA (*Cephaleuros mycoidea* KARST.)	572
X.	SUMMARY	573

I. INTRODUCTION

TEA is grown extensively in the hilly tracts of Palampur and Kangra Tehsils in the Punjab. The bulk of the tea, however, is grown in Palampur itself. Palampur has an elevation of about 4,300 ft. above sea level and lies at the foot of the Dhaula Dhar in the outer Himalayan range, commanding a beautiful view of the valley below. It gets a rainfall of about 100″ between March and October when the temperature is also sufficiently high. Tea plants cannot be grown in Palampur Tehsil beyond 5,000 ft. for it would not be sufficiently warm not below 3,000 ft. in that area for want of sufficient precipitation.

The cultivation of tea plants in Kangra Valley dates back to 1849, and from 1860 various tea plantations cropped up, mostly under British owners. The area under tea was approximately 10,000 acres at the beginning of the present century. But as the tea estates in North-East India developed, the effect of competition was severely felt by the Kangra Estates and many of the original owners and managers left the district. These tea estates passed into the hands of the people who took little interest in the development of these estates and who in many cases remained absentee owners. Most of the tea areas in the district were planted sixty to seventy years back and there has been great falling off in outturn, due also no doubt to a certain extent, to faulty processes of manufacture.

It is mostly green tea that is manufactured here, though a very small quantity of very poor quality of black tea is also being manufactured. The Kangra Valley green tea is exported to Kashmir, Afghanistan and Central Asia. Price of this green tea is less than 1/3rd of that of Kachar and Ranchi green tea. This deterioration has been due to faulty processes of manufacture and to utter neglect of proper cultivation. Plants are in many estates smothered by heavy growth of grass, practically no manuring is done, and often faulty methods of pruning and picking may be seen and, above all, insufficient attention is paid to pests and diseases. This has resulted in many diseases of root, stem and leaf, specially in the older bushes. Practically no new variety of tea has been introduced nor any new area planted during the last thirty years.

In this paper, the diseases of the tea bush from various tea gardens in Palampur have been studied by the authors during 1930-34. Many of these diseases, no doubt, could be got rid of by judicious and periodic pruning. Actually many diseases have been noted but the following only are described in this paper, *viz* :—

1. Grey blight caused by *Pestalozzia theae.*
2. Brown-spot disease of the tea bush caused by *Phoma theicola.*
3. Brown blight of tea caused by *Colletotrichum camelliae.*
4. Scabbing of leaves caused by the joint action of *Phoma*, *Pestalozzia* and *Colletotrichum.*
5. Copper blight caused by *Guignardia camelliae.*
6. Internal root disease caused by *Botryodiplodia theobromae.*
7. A disease of the stem caused by *Hendersonia theicola.*
8. Red rust of tea caused by *Cephaleuros mycoidea.*

II. GREY BLIGHT (*Pestalozzia theae* SAWADA)

The disease is of wide occurrence and causes considerable loss. Minute brownish spots first appear on the upper surface of older leaves. Many of these spots coalesce and considerable area of the leaves may be covered by the spots which later take up a greyish or whitish appearance and become surrounded by brownish margins (Plate XLIX). Spots also appear on the under-surface of the

PLATE XLIX

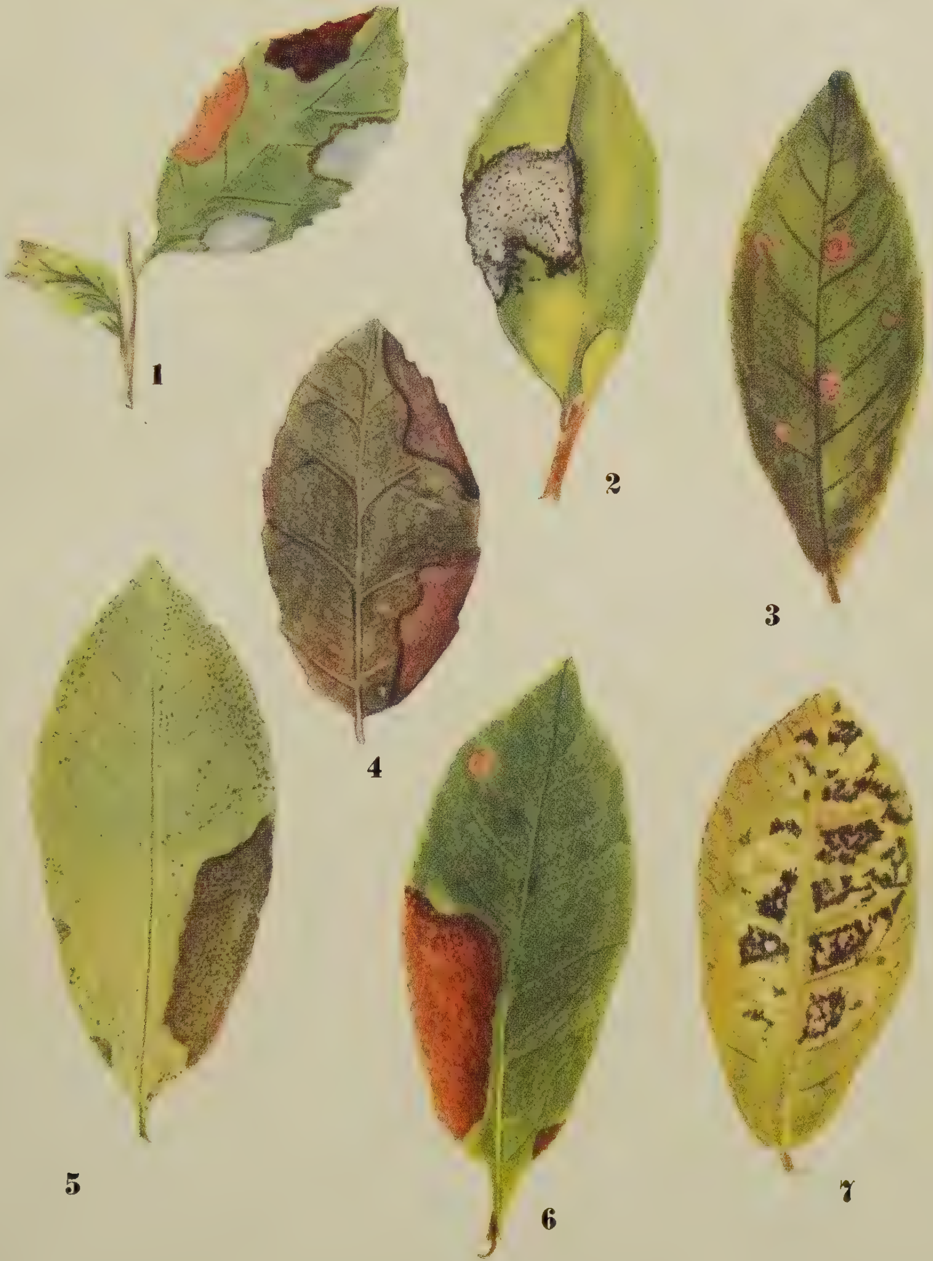
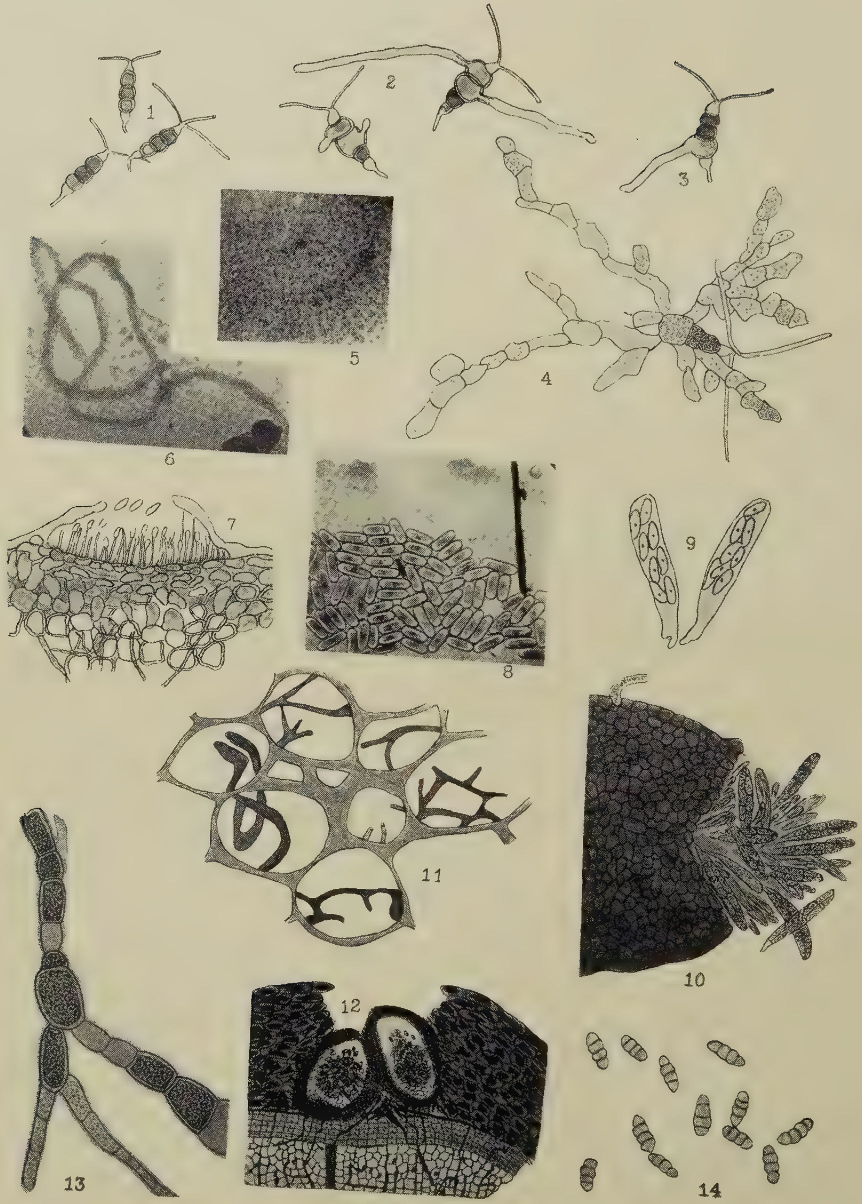


Fig. 1. Copper blight (*Guignardia camelliae*).
Fig. 2. Grey blight (*Pestalozzia theae*).
Fig. 3. Red rust (*Cephaleuros mycoidea*).
Fig. 4. Brown spot (*Phoma theicola*).
Figs. 5 & 6. Brown blight (*Colletotrichum camelliae*)—lower and upper surfaces.
Fig. 7. Scabbed leaf.

PLATE L



1. Spores of *Pestalozzia theae* (×215). 2-4. Germination of *Pestalozzia* spore (×225). 5. Photograph of a portion of culture dish showing pycnidia of *Phoma theicola* (×¼). 6. Photo-micrograph showing liberation of long coils mucilage containing spore from a pycnidium of *Phoma theicola* (×125). 7. Drawing of a section of a leaf showing an acervulus of *Colletotrichum camelliae* (×65). 8. Photo-micrograph showing spores and a seta of above (×150). 9. Two asci from the perfect stage of *Colletotrichum* (*Glomerella cingulata*) (×200). 10. Part of a perithecium *Guignardia camelliae* (×125). 11. Section of the wood showing internal hyphae *Botryodiplodia theobromae* (×215). 12. Section showing hyphae and two pycnidia of *Botryodiplodia theobromae* embedded in the cortex (×45). 13. Chlamydospores formation in *Botryodiplodia theobromae* (×215). 14. Spores of *Hendersonia theicola* (×200).

leaves, but these seldom turn grey. Pycnidia are found mostly in the spots on the upper surface and occur mostly along the margins of the patches. Older spots become very fragile and rolling of leaves may be seen in exceptional cases. In transverse sections of the diseased tissue inter-cellular hyphae may be seen. Leaf tissue in the infected spots become gradually disorganised. Pycnidia are sub-epidermal and bowl-shaped and measure 75-150 μ in diameter. Spores are borne on stalks along the inner lower wall of the pycnidium. Spores get detached along with the stalk. The stalk is unbranched ; 3·1 to 9·3μ in length. Spores are long, straight or slightly curved, fusiform, four septate and are slightly constricted at the septa and measure 23-35 × 4·65μ. The three middle cells are dark coloured (olivaceous) while the end cells are hyaline. The upper cell is crowned with three hyaline filiform appendages, the ends of which are slightly knobbed. The appendages are 6·2 to 40·3 μ in length. The appendages may sometimes be twice as long as the spores. On a random count of 100 spores from different pycnidia, the average length of the coloured cells, upper and lower hyaline cells, stalk and the appendages, has been found to be as follows :—

Coloured cells	16·6μ
Upper hyaline cells	5·2μ
Lower hyaline cells	4·2μ
Stalk cells	6·2μ
Appendage cells	16·1μ

The causal organism has been isolated and grown in pure culture from single pycnidiospores. Dense white colonies of the fungus developed on potato glucose agar at 25°C. in three days. In this medium, abundant pycnidia developed in a week's time. Freshly formed spores germinated readily. Older spores lose their viability soon. Only a small percentage of spores from two months old pycnidia germinated. The germination is peculiar in that the germ tubes are usually given out from the third lower coloured cell. On the absorption of water, it swells and by rupturing the wall the germ tube protrudes. Sometimes a number of germ tubes, up to five, may be seen coming out of the third lower coloured cell. Occasionally, the middle or the upper coloured cell may give out germ tubes (Plate L, figs. 1-4).

In culture, the nature of the growth of the fungus depends on the medium on which it is grown. In poor media vegetative growth is poor, but pycnidia are abundantly formed. In richer media the vegetative growth is luxurious but very few pycnidia are formed. By reducing the concentration of the medium, earlier and more abundant formation of pycnidia could be induced. Pycnidium formation has been found to be independent of light factor. Depending on the medium on which it is grown, pycnidia developed equally well both in light and in darkness. Under humid conditions more pycnidia are formed. This fungus has a rather high optimum temperature for growth, *viz.* 32°C. Though it can tolerate a very low temperature, no pycnidia are formed at a temperature below 18°C.

The authors have been able to establish the pathogenicity of the fungus by successfully inoculating cut leaves in Petri dishes, as well as plants growing in pots at Lahore. Pycnidia were formed on the leaves of inoculated plants in about three weeks' time.

III. Brown spot (*Phoma theicola* Petch)

Brown-spot disease is not so common in Palampur as the Grey blight (*Pestalozzia theae*) described above. It produces reddish brown patches on both the surfaces of the leaf on which minute dark coloured pycnidia are borne (Plate XLIX). The cells in the affected tissue become disintegrated in many places. Internal hyphae are profusely seen in sections of the infected spots. In its general character the disease is similar to the diseases caused by *Colletotrichum camelliae* (Brown blight) and also *Phoma camelliae.* It differs from the former in that the spores are formed in definite pycnidia and from the latter in the possession of a clear ostiole by the pycnidium.

Examination of hyphae in the host-tissue as well as from culture shows that these are hyaline, septate and branched. Oil globules are occasionally seen in the hyphae from the cultures. Spores are produced in pycnidia which are epiphylous and are found on both the sides. Spores are broadly oval, hyaline, non-septate, biguttulate $3{\cdot}2\text{-}12{\cdot}8\ \mu \times 1{\cdot}6\text{–}7{\cdot}8\ \mu$. Pycnidia are globose, $96\text{-}300\mu$ in diameter. Wall of pycnidia $25\text{-}60\ \mu$ thick. Presence of ostiole is a very characteristic feature. Spores ooze out through these ostioles. In cultures specially, pycnidia growing close together, fuse and often fused pycnidia with two or more ostioles may be seen.

Germination of spores was studied in glass cells. At 25°C. the spores germinated in about eight hours. Germ tubes are formed only from one side of the spores.

The fungus has been grown in culture on various media. Luxurious vegetative growth took place in Cook's medium and largest number of pycnidia was formed in corn-meal agar (Plate L, fig. 5). Pycnidium arises in a meristogenous manner. Pycnidium formation is independent of light but requires the presence of free air . Under humid conditions spores embedded in long coils of mucilage are seen to come out through the ostioles (Plate L, fig. 6). Chlamydospores have been found to develop only in very old cultures.

Inoculation experiments were carried out in Lahore with cut leaves and potted plants only. Spore suspensions placed on marked areas on both upper and lower surfaces, produced infection. Those areas became discoloured in a week's time and later typical pycnidia developed on those areas. These inoculation experiments proved the pathogenicity of the fungus beyond doubt.

IV. Brown blight (*Colletotrichum camelliae* Massee)

It is one of the most destructive and dangerous parasite of the tea plants. It causes greatest danger to younger leaves and also attacks the stem.

The diseased spots are at first yellowish green. As they grow older and increase in size, they become dark brown and ultimately turn chocolate brown (Plate XLIX). In extreme cases, they turn almost black. When a diseased leaf is held against light, a yellowish band is sometimes seen round the affected spots, due to the spreading of the mycelium into the healthy tissue, which gradually loses its green colour. The fructifications mostly occur on the under-surface of the leaves as minute black points scattered over the spot, often with a tendency to group themselves along the veins.

Section of diseased leaf tissue shows the presence of hyphae ramifying in all directions. If it includes an acervulus, the hyphae are seen to collect and form a thick mass of pseudo-parenchyma on which erect hyphae bearing spores at their tips are borne. These acervuli appear pinkish and are irregularly arranged. Dark setae are scattered round the acervuli (Plate L, fig. 7). The conidia are hyaline, cylindrical, straight or slightly curved, continuous with obtuse ends and in a mass they appear pinkish. Though almost hyaline, the conidia present a slight greenish tinge at certain stages of their growth, when examined under the microscope. Conidia measure 10-21 × 3·5-5 μ (Plate L, fig. 8). Spores germinate in eight to ten hours and germ tubes from both ends may be produced. Setae arise from the base of the acervulus. They are generally few, dark brown, bristle-like and sometimes slightly curved. In a dry substratum, usually more setae develop. They measure 33-83 × 4 μ.

In cultures black pin-head-like structures appear. These are formed by compact masses of dark hyphae aggregating and forming closely-knit masses and remain half submerged in the medium in which they are formed. These black bodies (stroma) appear specially in starchy media. Most probably these are resting bodies. The fungus has been grown in a number of different media. Most luxuriant vegetative growth was found in media containing sugar (glucose or sucrose).

The pathogenicity of the fungus has been established by the authors by making artificial inoculations on cut leaves as well as on plants in pots.

The perfect stage of the fungus (*Glomerella* stage) has been obtained on artificially inoculated leaves, but has never been obtained in cultures. The dark brown perithecia found on the inoculated leaves were pear-shaped with a short neck. These measured 126μ in diameter. The asci are hyaline, sessile, and clavate and measure up to 72 μ in length (Plate L, fig. 9). The ascospores are eight in number, hyaline, slightly curved, and measure 12·6-16·2 × 3·41 μ. The ascospores readily germinated in water, but though these were grown in various media, no perithecia were developed in any of these cultures—only acervuli of the *Colletotrichum* form were produced.

V. SCABBING OF LEAVES

Scabbed appearance is due to infection of the leaves by several fungi. Only mature leaves show scabbing (Plate XLIX). At first yellowish green spots are formed, which later turn blackish brown and finally black. The spots are rather irregular-angular in outline. The different parts of the same spot may show different colorations, giving rise to a sort of mottled appearance. Sometimes several spots may coalesce and the whole leaf may appear scabbed. These leaves become very brittle.

From the scabbed leaves the following fungi have been isolated, *viz* :—

Pestalozzia theae Sawada
Phoma theicola Petch, and
Colletotrichum camelliae Massee.

Of the above, at least two are always found in scabbed spots and usually *Phoma* is always found in association with either *Pestalozzia* or *Colletotrichum*. The spores of *Pestalozzia* and *Colletotrichum* showed usual measurements but those of *Phoma* were ordinarily smaller in the scabbed leaves. When however these were isolated and grown in different culture media, no variations in spore measurements from the normal were found.

Scabbed symptoms have been produced by inoculating leaves with mixed cultures of the above fungi.

VI. COPPER BLIGHT (*Guignardia camelliae* (CKE.) BUTLER*)

The symptoms are very similar to those of Brown spot (*Phoma theicola*). The spots usually later become larger and extend to the margins of the leaves. The spots on the upper surface are at first yellowish brown but later become copper coloured and finally grey, resembling Grey blight (*Pestalozzia theae*). The spots are circular in outline at the beginning but later become irregular in shape. When the spots turn grey, there is a sharp demarcation formed between the diseased and the healthy portions of the leaves. The under-surface of the spots show a greyish brown coloration.

On the diseased spots minute black perithecia of *Guignardia* are formed. The spots are extremely brittle and bear numerous minute cavities formed by the disintegration of the epidermal cells.

The fungus was grown in culture from the hyphae in the host tissue and obtained pure by Brown's hyphal tip method. In cultures, it readily produced typical perithecia.

---

* This combination is not found in Butler and Bisby's 'Fungi of India' but is mentioned by Petch in his 'Diseases of the Tea Bush'.

The hyphae may be seen inside the host tissue, specially before fructifications are produced. The measurement of the cells of the hyphae varies greatly.

The width of the hyphae varies between 2·4 and 8 μ and the length of the cells 9·6 to 80·8 μ. Older hyphae become additionally septate. Cell fusions are of common occurrence. Sclerotia-like bodies are formed in abundance in all starchy media. Hyaline cylindric conidia, non-septate, with obtuse ends are formed abundantly on aerial hyphae on agar cultures, specially on Czapek's and Cook's. Conidia measure 9·4-34·3×3·2-6·4 μ. Perithecia developed in all agar media. Though almost colourless at first, these soon take up a light brown coloration and finally become dark brown. Mature perithecia from cultures measure 109-274 μ in diameter. On host tissue, they seldom grow bigger than 250 μ. The asci are elongate, elliptical and eight-spored (Plate L, fig. 10). A mature ascus measures about 60 μ in length. The spores are eliptic, hyaline and continuous and are arranged in two rows. Spores measure 17·6-20·8×5·6-7·2μ.

Spores germinate by producing a beak which soon bursts and a hyaline germ tube is given out. It branches and conidia are formed in about four days. Sometimes a conidium may be borne on germ tube within fourteen hours. Though the conidia germinated so readily, the ascospores failed to germinate at all in any media or temperature tried so far.

Cut leaves and leaves of potted plants were successfully inoculated at Lahore with conidiospores. The fungus is a wound parasite and attacks only the leaves. The disease spreads quickly when the temperature is high.

VII. Internal root disease (*Botryodiplodia theobromae* Pat.)

The causal organism grows on a number of host plants other than tea. It can attack unwounded and quite vigorously growing trees. This root disease comes into prominence only after pruning is done. After pruning, the infected plants either fail to produce any new shoots and die off completely, or a few leaves that may be produced become mottled with patches of yellow and later turn black before they die off. Inoculated plants invariably showed the latter kind of symptoms.

Examination of the roots of dying and dead plants showed no external hyphae. But sections of the roots always showed the presence of dark brown or almost black fungal hyphae with thick walls (Plate L, fig. 11).

On the dead twigs, pycnidia of *Botryodiplodia theobromae* are found (Plate L, fig. 12). Cultures were made from pycnidiospores. The study of the cultures shows that the hyphae are hyaline at the beginning which later turn almost black. The width of the hyphae varies from 2·4 to 8 μ, the cells of the hyphae measure 9·6×41·6 μ or more. Fusion between cells of hyphae leading to a net-like structure is quite common. Chlamydospores are formed in culture (Plate L, fig. 13). The hyphae have a tendency to fuse with one another along the whole length and several hyphae may so fuse forming a dark rhizomorphic cord. Pycnidia are formed regularly. Hyphal knots are formed just beneath the under-surface of the cortex which later develop to pycnidia. In culture both aerial and submerged pycnidia are formed. These are globose or flask-shaped, black carbonaceous and have distinct outer and inner walls. From the inner surface, hyaline cylindrical conidiophores arise. The spores are hyaline and continuous when young but later become one-septate and dark coloured. The diameter of the pycnidia varies from 280·6 to 561·2 μ and the spores measure 19·2-25·6× 7·2–11·2 μ.

Stromata consisting of mass of rhizomorphic cord are also regularly formed. These measure 1·5 to 4·0 mm. in diameter.

Spores germinate readily. A beak-like process is first formed, which later ruptures, giving out a hyaline germ tube.

Inoculation experiments with potted plants at Lahore proved definitely the fungus to be a strong parasite. All soil inoculations succeeded and aerial parts incised with a lancet needle and inoculated with spore suspensions, or plants simply sprayed with spore suspensions after being pruned, quickly developed the symptoms of the disease and the plants died in six to eight weeks' time. It is probable that the sudden prominence of the disease after pruning is due to the fact that through all the wounds produced at the time of pruning, the plants get severely infected by the pycnidiospores which ripen at that time.

VIII. BARK DISEASE (*Hendersonia theicola* CKE.)

It has been found to cause only minor damage to tea bush in the Kangra Valley. The affected stems become blackish, rough and rugged. On these spots, hyphae and spores of the causal fungus are plentifully found. The spores are pale-brown in colour, tri-septate, narrow oval or sub-cylindrical, slightly constricted at the septa and rounded at the ends. The spores measure 10-13×3·5-4·5 μ (Plate L, fig. 14). On germination germ tubes may be formed from any cell of the spore. The view that *Hendersonia* is an immature form of *Pestalozzia* has been expressed by several authors. From the cultural studies of the fungus, this view is not at all supported by the authors.

IX. RED RUST (*Cephaleuros mycoidea* KARST.)

The Red-rust disease is not so common in North-West India, as in North-East India. It has however been noticed on tea plants near about Baijnath Paprola. It is caused by an alga, *Cephaleuros mycoidea*. On the upper surface of the leaves, reddish brown disc-like spots are formed (Plate XLIX). Stem infections have not been observed so far. The reddish discs, though superficial, are firmly attached. On these red patches erect orange-coloured filaments—the sporangiophores—are borne. The host tissue below the discs become discoloured and later the cells become disintegrated, and the patches which formed elevated spots at the beginning, may actually become depressed.

The thallus of the alga forms a cushion-like structure of radiating filaments from a central point. On these patches, during the rainy season sporangiophores bearing sporangia are developed. Due to dense formation of orange-coloured sporangiophores, these patches become very prominent. A number of sporangia are borne on each of these sporangiophores. There are also sterile hairs present along with the sporangiophores. The mature sporangium as a whole, breaks off from the stalk and, if conditions are favourable, they form zoospores which bring about infection of new spots of leaves and of new plants.

This causal organism is not a parasite in the ordinary sense as it does not penetrate the host cells, but derives nourishment by virtue of the high osmotic value of its cells.

X. SUMMARY

Various diseases of the tea bush from the tea gardens in Palampur (Kangra) have been studied by the authors. Of these the following diseases have been described in the paper :

1. Grey blight (*Pestalozzia theae*).
2. Brown-spot disease (*Phoma theicola*).
3. Brown blight (*Colletotrichum camelliae*).
4. Scabbing of leaves (combined effect of *Phoma*, *Pestalozzia* and *Colletotrichum*).
5. Copper blight (*Guignardia camelliae*).
6. Internal root disease (*Botryodiplodia theobromae*).
7. A stem disease (*Hendersonia theicola*).
8. Red rust of tea (*Cephaleuros mycoidea*).

The causal organisms have been isolated and grown in culture and their characteristics on the host plants as well as on various culture media have been described. Inoculation experiments have been performed in all cases to establish the pathogenicity of the organisms. Of the diseases described, the Grey blight (*Pestalozzia theae*) and Brown blight (*Colletotrichum camelliae*) are responsible for the greatest loss in this area.

STUDIES ON THE ROOT-ROT DISEASE OF COTTON IN THE PUNJAB

IV. THE EFFECT OF CERTAIN FACTORS INFLUENCING INCIDENCE OF THE DISEASE

BY

R. SAHAI VASUDEVA, B.Sc., Ph.D. (Lond.), D. I. C. (Lond.)
Assistant Cotton Mycologist, Department of Botany, Agricultural Research Institute, Lyallpur

(Received for publication on 15th December 1936)

(With four text-figures)

CONTENTS

	Page
I. Introduction	575
II. Experimental	576
(A) Effect of irrigation on the incidence of the root-rot disease	576
(B) Relation of soil moisture to the incidence of the disease	577
(C) Effect of soil moisture on infection	579
(D) Periodicity in the parasitic activity of the causal fungi	580
(E) Prevalence of the disease in different tracts	585
(F) Effect of sowing cotton on 'ridged' and 'flat' rows combined with the effect of sowing date	586
III. Summary	587
References	587

I. Introduction

It has been observed that the amount of moisture in the soil seems to influence greatly the incidence of the cotton root-rot disease in the Punjab. It has also been found that if the time of sowing of cotton is varied there is a considerable difference in the mortality of plants in infected fields. The sowing time of cotton can be extended from the middle of April to middle of June or even to a later date. There is a definite decline in the percentage of affected plants in late-sown crop.

The results indicate a tendency towards periodicity in the parasitic activity of the causal fungi [Martin, 1936]. With a view to determining exactly the bearing of these factors on the intensity of attack of the disease, experiments reported in this paper were carried out.

II. Experimental

The land used for the experiments was either that which was naturally and consistently infected or the plots were inoculated heavily with cultures of a fungus provisionally called *Rhizoctonia bataticola** and *Rhizoctonia solani* Kuhn group (new strain) in order to secure conditions most suitable for obtaining reliable and comparable data.

The incidence and the progress of the disease were studied from week to week in a large number of plots. The actual position of the infected plants was marked and the rate of progress of the disease recorded throughout the season for several years. Outline maps of the affected plots were prepared indicating the patches where the plants were killed by the disease.

(*A*) *Effect of irrigation on the incidence of the root-rot disease*

For this experiment a piece of land situated in the Botanical Area at Lyallpur in which the disease is known to occur in a very severe form year after year, was selected and divided into plots. These plots were randomized and grouped into three equal lots. Non-experimental strips were left out on all sides of the experimental plots in order to avoid the effect of irrigation from plot to plot. *Gossypium indicum* var. *Mollisoni* (No. 15) was sown in rows three feet apart and the number of plants kept in each plot was almost the same.

All the plots were irrigated about six weeks after sowing. After this they were irrigated throughout the season as required, each group of plots receiving frequent, less frequent and very few irrigations respectively as given in Table I. These irrigations were given in addition to the rainfall during the experiment. The first experiment was conducted in 1933. A similar experiment was repeated during 1934, 1935 and 1936. The results of the irrigation experiments carried out during these years are summarised in Table I. During the years 1933 and 1934 each treatment (irrigation) had five replications but during 1935 there were only four.

---

*The position regarding specific name of the fungus has not yet been determined. It will be given in a later publication.

TABLE I

Root-rot mortality in relation to the number of irrigations

Date of sowing	No. of irrigations	Total No. of plants	Plants killed due to root-rot	Per cent mortality
24th April 1933	5	284	93	32·39
	3	298	93	31·20
	2	294	67	22·78
30th April 1934	8	437	190	43·47
	4	472	186	39·40
	3	460	177	38·47
19th May 1935	7	352	282	80·11
	4	360	205	56·94
	3	360	181	50·27
8th May 1936	6	326	98	30·06
	3	324	93	28·70
	1	332	84	25·30

The results obtained indicate that when fewer irrigations are given the incidence of the disease is retarded. These results are quite marked in the data obtained during the years 1933 and 1935. The results obtained during 1934 and 1936 also give similar indications though the differences are not so marked.

It was, however, noticed that an adequate supply of moisture in the early part of the season, when the plants are about six weeks old, favours the development of the disease and within a fortnight the mortality reaches its optimum.

(*B*) *Relation of soil moisture to the incidence of the disease*

One of the infected plots in the Botanical Area at Lyallpur was selected for this experiment. The soil moisture in this plot was determined frequently from the time of sowing cotton (April) till the time when the disease almost ceased (September). Along with soil-moisture determinations which were carried out in one-foot layers up to a depth of five feet, records of the incidence of the disease in the plot were taken throughout the season. The soil-moisture determinations were carried out before and after irrigation and the incidence of the disease recorded on the same days. For estimation of moisture, soil samples from different parts of the same field were taken in order to get a fairly representative idea of the moisture content of the whole field. Before sowings the field was carefully levelled in order to avoid uneven distribution of irrigation water in any part of the field.

A study of the records of the experiment carried out in 1936 shows that throughout the season percentage mortality due to root-rot corresponds to the percentage of soil moisture in the field. Fig. 1 shows the relation of soil moisture to mortality due to root-rot. The soil-moisture curve shows total amount of soil moisture in five-feet layer of the soil. The soil-moisture curve runs almost parallel to the mortality curve throughout the season except on the 16th of June. Almost identical curves for soil moisture are obtained if they are drawn for one-foot layers of soil moisture on particular dates.

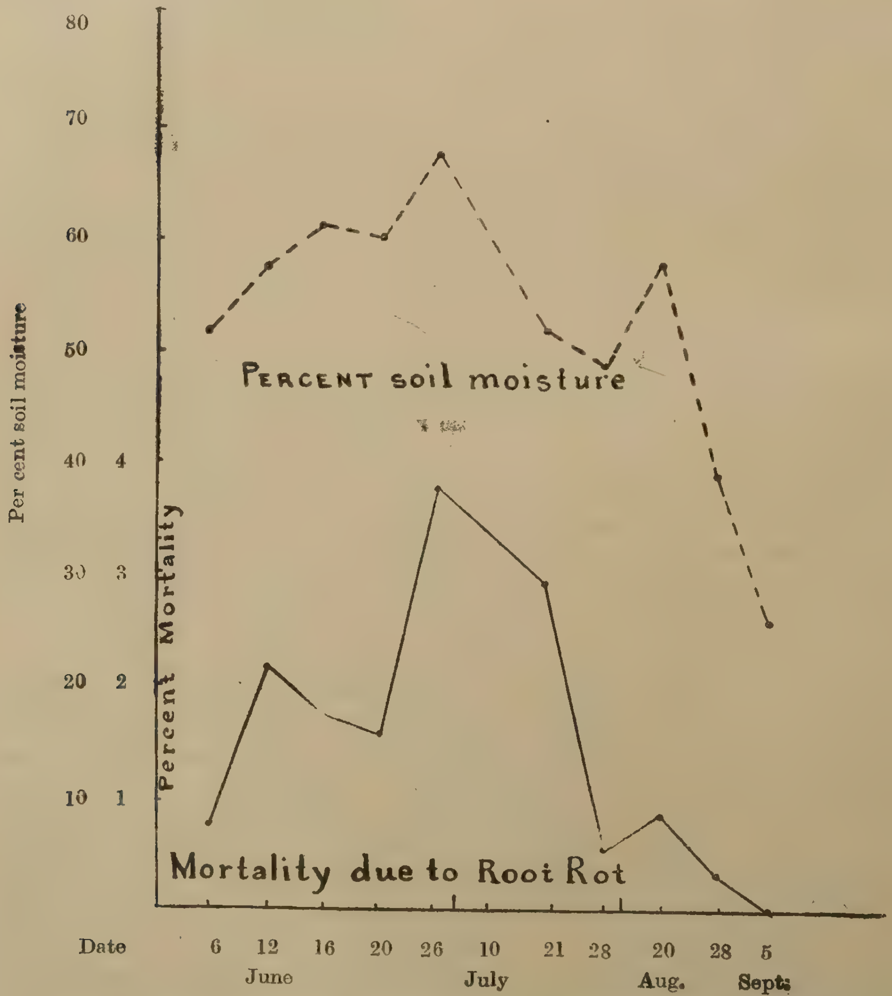


FIG. 1. Showing the relation of soil moisture to mortality due to root-rot.

(*C*) *Effect of soil moisture on infection*

Infection experiments were conducted in the laboratory in order to determine the effect of soil moisture. The plants used for inoculations in these experiments were grown in glass jars containing sterilized soil. The jars were divided in four lots and the soil moisture in each lot adjusted at five, ten, fifteen and twenty per cent. During growth the soil moisture was maintained uniformly in each group of jars by adding water through glass tubes inserted in the soil. The containers were weighed after every twenty-four hours and necessary water added to make up the loss by evaporation and transpiration. To facilitate irrigation a certain amount of gravel was added in each jar before filling it with the soil. The soil which was added over the gravel was air dried, thoroughly mixed until quite uniform and mixed with measured quantities of water to make up to the required percentage of soil moisture. Covers made of grease-proof paper were used to control undue loss of moisture from the soil surface.

In one of the experiments metallic pots with small holes in the sides near the bottom were used as containers in place of glass jars.

The plants were inoculated with *R. bataticola* and *R. solani*. The inoculated pots were kept in the glass-house and the temperature was maintained between 15° and 31°C. by putting up heaters*. Germination was very poor in the jars containing five per cent soil moisture and thus only a few plants were available for inoculation purposes. The results of such inoculation experiments are given in Table II.

TABLE II

Effect of soil moisture on infection

Fungus	Experiment		10 per cent soil moisture			15 per cent soil moisture			20 per cent soil moisture		
			Total number of plants			Total number of plants			Total number of plants		
	No.	Date	Inoculated	Wilted	Per centage mortality	Inoculated	Wilted	Percentage mortality	Inoculated	Wilted	Percentage mortality
R. bataticola . .	1	10-12-35	10	3	30·00	10	10	100·00	8	7	87·50
	2	2-1-36 .	7	2	28·57	2	2	100·00	7	6	85·71
R. solani . .	1	21-2-36 .	11	6	54·54	13	11	84·61	10	10	100·00
	2	27-3-36	12	2	16·66	13	4	30·76	14	7	50·00
	3	5-4-36 .	19	3	15·78	18	4	22·22	10	7	70·00

The data given above show that *R. bataticola* and *R. solani* have caused heavy infection at fifteen per cent and twenty per cent soil moisture. Inoculations carried out at five per cent soil moisture gave evidence of the inability of both the organisms to parasitise and they failed to produce any symptoms of the disease. Controls kept in corresponding percentages of soil moisture always remained healthy.

---

* As required for the experiment.

(*D*) *Periodicity in the parasitic activity of the causal fungi*

As already reported the attack starts in June and within a fortnight the maximum percentage of attacked plants is reached. It, however, continues to be vigorous throughout July. In August the death rate of plants gradually declines and the disease almost ceases by the end of September.

The course of the disease shows that the causal organisms, *R. solani* and *R. bataticola*, are most active and cause maximum damage during June and July. Having known the time when the infection is at its highest it was thought interesting to investigate the effect of sowing cotton late when major portion of the period of optimum activity of the causal fungi is over. For this purpose a preliminary experiment was carried out in 1934. Two naturally affected plots were selected in the Botanical area at Lyallpur and each was divided into nine sub-plots. Sowings were made in randomized plots at intervals of about a month from beginning of May to the 20th of June. In one set of nine sub-plots *desi* (Mollisoni 15) and in the other set American type 4-F (*Gossypium hirsutum*) was sown.

The results of this experiment are given in Table III.

TABLE III

Effect of time of sowing on the virulence of the disease

Date of sowing	Variety	Plot No.	Total No. of plants in the beginning of season (3 sub-plots)	Total number killed by root-rot	Plants recovered	Percentage of plants killed	
1st May 1934 .	4-F	1	234	13	1	5·1	Av. 5·60
,, .	4-F	2	246	12	1	4·5	
,, .	4-F	3	250	18	..	7·2	
1st June 1934 .	4-F	4	177	39	5	19·2	Av. 19·16
,, .	4-F	5	164	32	5	16·5	
,, .	4-F	6	225	51	2	21·8	
20th June 1934 .	4-F	7	244	0	..	0·0	Av. 2·43
,, .	4-F	8	248	8	..	3·3	
,, .	4-F	9	250	10	..	4·0	
1st May 1934 .	Mollisoni (15).	1	250	11	..	4·4	Av. 4·46
,, .	,,	2	241	15	..	6·2	
,, .	,,	3	250	7	..	2·8	
1st June 1934 .	,,	4	238	19	..	8·0	Av. 7·66
,, .	,,	5	245	21	..	5·6	
,, .	,,	6	245	23	..	9·4	
20th June 1934	,,	7	250	0	..	0	Av. 0·26
,, .	,,	8	250	0	..	0	
,, .	,,	9	250	2	..	0·8	

The examination of the data given above clearly shows that the plots of cotton both of *desi* and American varieties when sown on the 20th of June suffered least from the disease and that the number of plants killed due to root-rot in each plot was comparatively much smaller than in the plots sown on earlier dates.

A similar experiment was repeated in 1935 both at Lyallpur and the B. C. G. A. Farm, Khanewal. Plots uniformly affected with the disease had been selected during the previous year. Both *desi* and American cottons were tested. The experiments were carried out in randomized plots in triplicates.

The results of two such experiments carried out at Lyallpur and the B. C. G. A. Farm, Khanewal are summarized in Tables IV and V respectively.

TABLE IV

Effect of sowing date on the incidence of root-rot disease as observed at Lyallpur

Sowing date	Variety	Total No. of cotton plants (3 sub-plots)	Total number of plants killed	Per cent mortality
2nd May 1935	4-F	596	90	15·1
31st May 1935 . . .	4-F	526	36	6·8
29th June 1935 . . .	4-F	543	0	0
2nd May 1935 . . .	Mollisoni (15)	587	29	4·9
31st May 1935 . . .	,,	586	77	13·1
29th June 1935 . . .	,,	539	4	0·74

TABLE V

Effect of sowing date on the incidence of root-rot disease as observed at the B. C. G. A. Farm, Khanewal

Sowing date	Variety	Total number of cotton plants (3 sub-plots)	Total number of plants killed	Per cent mortality
14th April 1935 . . .	4-F	540	47	8·7
12th May 1935 . . .	4-F	540	81	15·0
15th June 1935 . . .	4-F	540	11	2·0
14th April 1935 . . .	Mollisoni (15)	535	58	10·8
12th May 1935 . . .	,,	540	91	16·8
15th June 1935 . . .	,,	526	10	1·9

Both these experiments conducted at Lyallpurand Khanewal show that the disease is reduced considerably in the crop sown on the 29th and 15th of June respectively. These results confirm the results obtained in 1934.

The experiments conducted in 1934 and 1935 gave indications that late sowing may be a means of evading losses due to root-rot. The investigation was followed up along these lines during 1936 by carrying out further tests. A plot of land in the Botanical area at Lyallpur and two plots at the B. C. G. A. Farm, Khanewal which are heavily infected with the disease were used for these experiments.

At Lyallpur the experiment was conducted in plot 46/23 which is very heavily infected and the mortality records of previous years show eighty to ninety per cent losses due to root-rot disease. This plot has been continuously under cotton for the last thirteen years without the addition of any manure so that the land is extremely poor. The plot was divided into forty-four sub-plots and *desi* (Mollisoni 15) cotton was sown on randomized system from 31st of March to 4th July. As far as possible the sowings in May and June were carried out at weekly intervals. Altogether, sowings were done on eleven different dates and four plots were sown each time. The results of the experiment are given in Table VI. (Fig. 2 shows the relation of sowing date to mortality due to root-rot at Lyallpur.)

TABLE VI

Incidence of disease in relation to sowing date at Lyallpur

Sowing date	Total number of plants (4 plots)	Plants killed by root-rot	Per cent mortality
31st March 1936	427	251	58·78
14th April 1936	446	297	64·34
28th April 1936	445	364	81·79
7th May 1936	459	391	85·18
14th May 1936	408	317	77·69
21st May 1936	506	282	55·73
29th May 1936	424	313	73·81
8th June 1936	512	226	44·14
16th June 1936	510	139	27·25
27th June 1936	686	126	18·36
4th July 1936	629	51	8·10

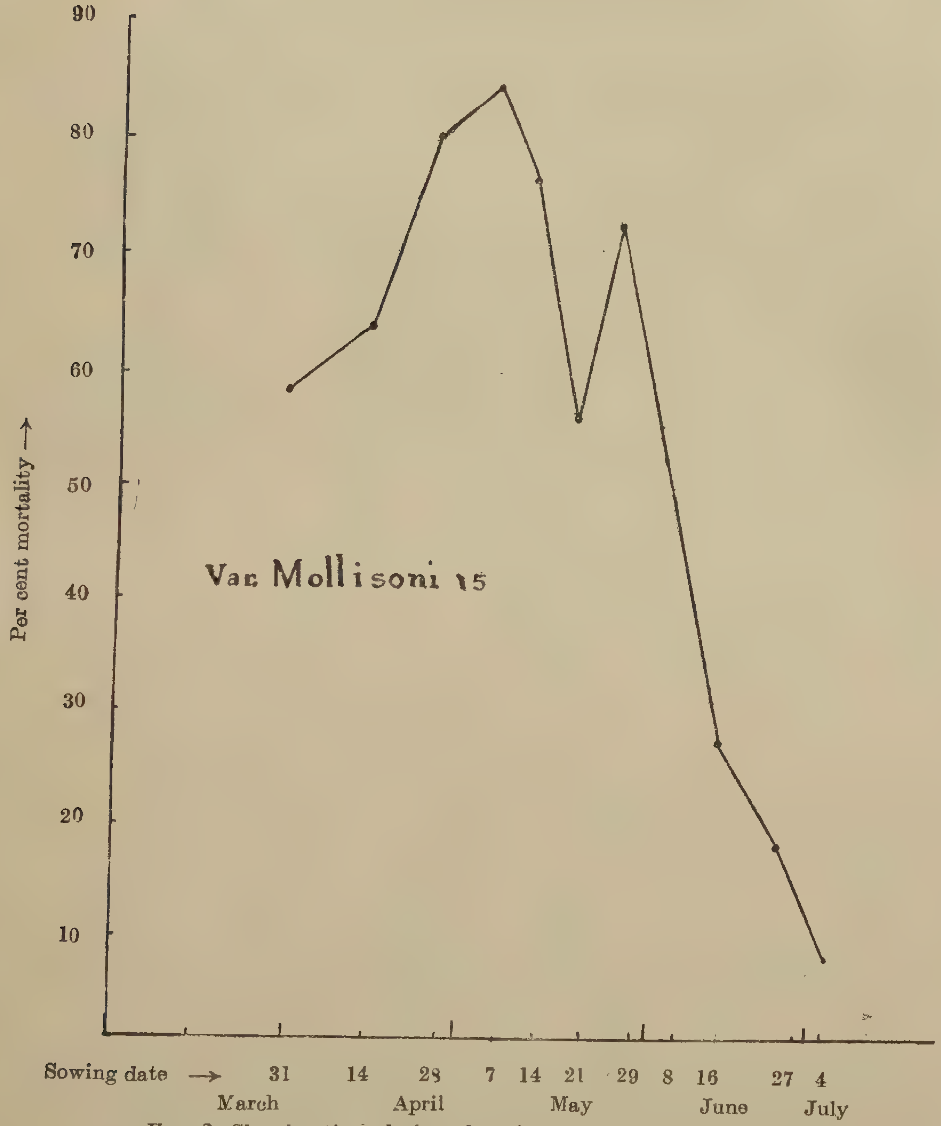


FIG. 2. Showing the relation of sowing date to mortality due to root-rot in plot 46/23 at Lyallpur.

It is clear from the above results that maximum damage is caused by the root-rot fungi in May-sown crop, whereas there is a regular reduction in the loss due to root-rot in sowings carried out after May.

Similar experiments conducted at the B. C. G. A. Farm, Khanewal, both with American and *desi* cottons, gave identical results. The data of Khanewal experiments are summarized in Table VII. Figs. 3 and 4 show the effect of sowing date on mortality as observed at Khanewal.

TABLE VII

Incidence of disease in relation to sowing date at the B. C. G. A. Farm, Khanewal

American (4-F)				*Desi* (Mollisoni 15)			
Sowing date	Total No. plants (4 plots)	Plants killed by root-rot	Per cent mortality	Sowing date	Total No. plants (4 plots)	Plants killed by root-rot	Per cent mortality
14th April 1936 . .	333	80	24·02	15th April 1936 . .	420	41	9·76
24th April 1936 . .	336	72	21·43	23rd April 1936 . .	420	54	12·85
7th May 1936 . . .	336	208	61·91	...	...	...	...
14th May 1936. . .	336	115	34·22	16th May 1936. .	420	168	40·00
21st May 1936 . . .	336	184	54·75	...	...	...	...
26th May 1936 . .	336	99	29·46	1st June 1936 . . .	420	72	17·14
4th June 1936 . . .	336	63	18·75	7th June 1936 . . .	420	114	27·13
12th June 1936 . .	336	61	18·15	15th June 1936 . .	520	42	8·07
18th June 1936 . .	336	53	15·77	21st June 1936 . .	420	12	2·85
25th June 1936 . .	336	15	4·46	...	...	...	...

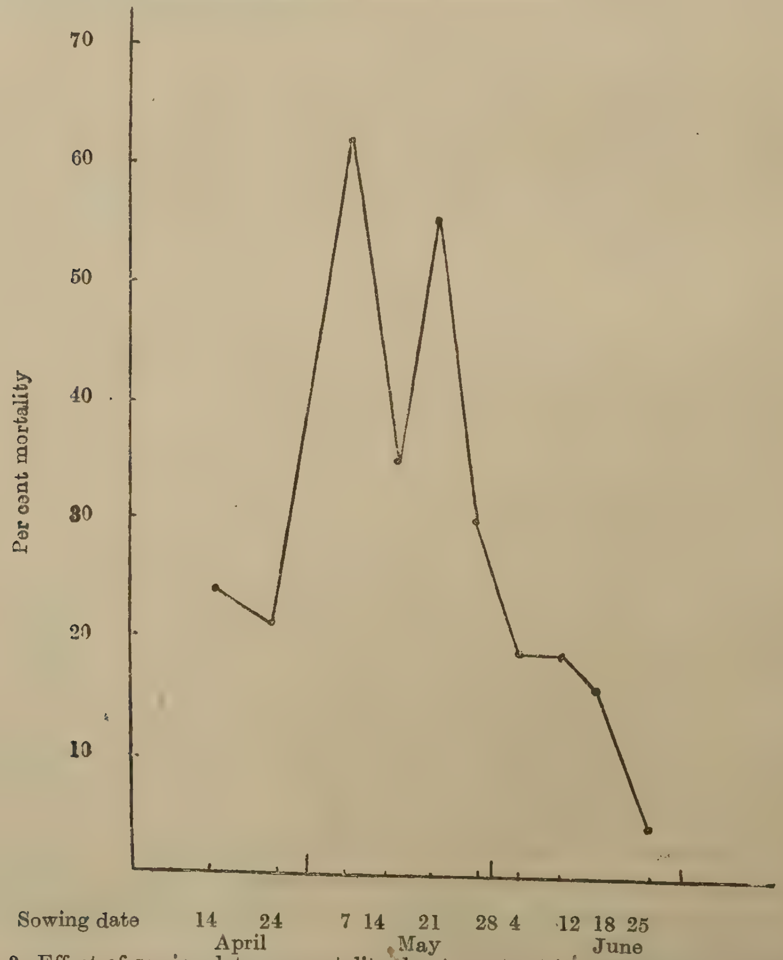


FIG. 3. Effect of sowing date on mortality due to root-rot in American cotton at Khanewal

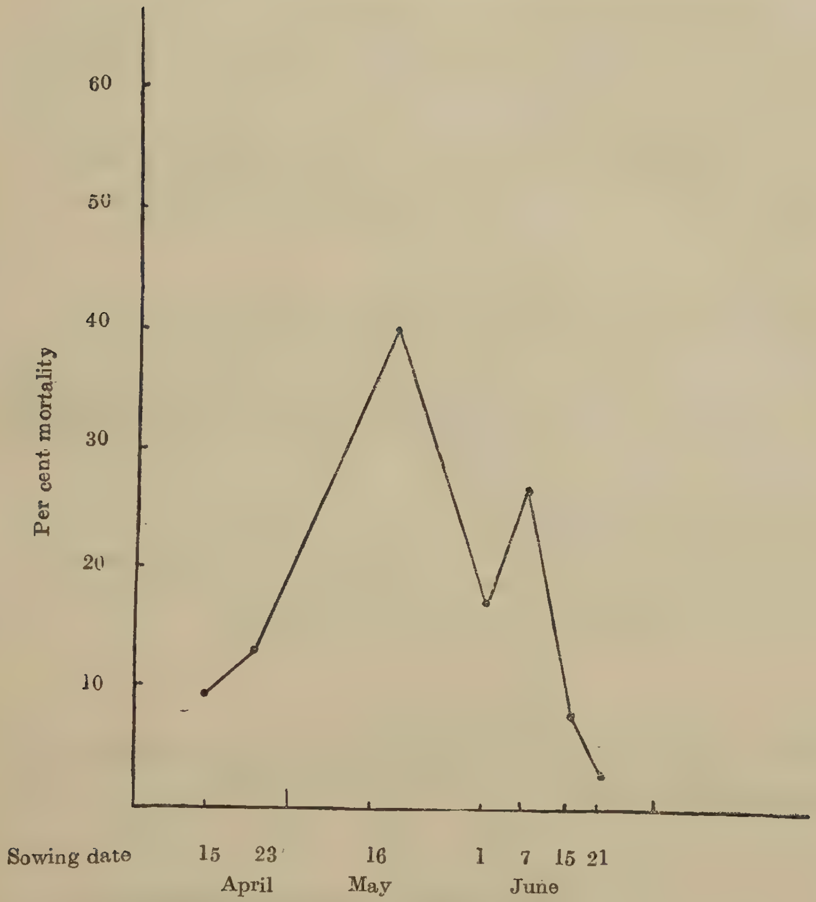


FIG. 4. Effect of sowing date on mortality due to root-rot in *desi* cotton at Khanewal

(*E*) *Prevalence of the disease in different tracts*

Root-rot disease has not been observed in cottons grown in '*barani*' un-irrigated areas, *i.e.* Ambala district, etc. These are all indigenous types. The same types in irrigated tracts are affected by the disease. Observations made at Ambala and the surrounding localities showed no diseased patches but the causal organisms were present in roots of cotton plants in many fields. Cultures prepared from the diseased roots yielded fungi identical to those of typical root-rot. A thorough examination of the roots of the plants obtained from Ambala district showed that the roots of the plants had been partially attacked but the disease had not advanced to a stage so as to cause wilting and ultimately the death of the entire plants. It shows that the conditions for an active parasitic attack in that locality are not favourable. The disease makes a start but the conditions being unfavourable organisms fail to develop in a virulent form. Cotton sowings in that tract of the province are carried out very late in the season (somewhere about the beginning of July) and may be that the conditions for the root-rot fungi to parasitise are rendered unfavourable during that season as already shown by the experiments conducted both at Lyallpur and Khanewal. Further investigation is required to work out just what exactly those conditions are which hinder the progress of the organisms in that tract.

(*F*) *Effect of sowing cotton on 'ridged' and 'flat' rows combined with the effect of sowing date*

Two plots situated side by side and infected with the disease to the same extent were used for this experiment. One of the plots was manured with farmyard manure at the rate of one thousand maunds per acre about one month before the first sowing and the other was left unmanured.

The experimental plots were divided into nine sub-plots each. American (4-F) cotton was sown in each sub-plot in alternate rows of 'ridged' and 'flat'. The sub-plots were randomized and sown in triplicates in April, May and June. The results of such an experiment are summarized in Table VIII.

TABLE VIII

Effect of 'ridged' and 'flat' sowings along with sowing date on the incidence of the disease (manured as well as unmanured plots)

Sowing date	Treatment	'Ridged' sowings			'Flat' sowings		
		Total number of plants	Plants killed by root-rot	Per cent mortality	Total number of plants	Plants killed by root-rot	Per cent mortality
15th April 1935	Manured	300	57	19·0	240	46	19·2
13th May 1935	,,	300	98	32·6	238	63	26·4
15th June 1935	,,	300	14	4·6	240	3	1·2
15th April 1935	Unmanured	298	93	31·2	238	73	30·6
13th May 1935	,,	298	141	47·3	240	79	32·9
15th June 1935	,,	300	14	4·6	240	11	4·6

It is clear from the data obtained that (*i*) there is no appreciable difference in the incidence of the disease when cotton is sown on 'ridges' or 'flat', (*ii*) the disease is considerably reduced in the crop sown on 15th June 1935, and (*iii*) there are indications that the disease is reduced to some extent in the manured plots.

Further work is required to test the effectiveness of organic manures and other factors affecting soil fertility.

I wish to place on record here my gratitude to Professor J. C. Luthra, I. A. S., for his interest and valuable criticism. Thanks are also due to Ch. M. Ashraf, Agricultural Assistant, and Mr. Hans Raj Sikka, Fieldman, for the help rendered. I desire to express my indebtedness to Mr. W. Roberts, C.I.E., Managing Director of the B. C. G. A., for the facilities offered at his Farm at Khanewal.

These investigations were carried out in connection with the scheme financed by the Indian Central Cotton Committee.

III. SUMMARY

(1) The bearing of soil moisture on the incidence of the root-rot disease of cotton has been shown.

(2) *Rhizoctonia solani* and *Rhizoctonia bataticola* cause heavy infection at fifteen per cent and twenty per cent soil moisture.

(3) Evidence of periodicity in parasitic activity of the causal fungi has been correlated with the time of sowing, showing thereby that the virulent attack of the disease may be evaded by skipping over the period of optimum activity of the causal organisms.

(4) There is no significant difference in the incidence of the disease when cotton is sown on ' ridges' or ' flat '.

REFERENCES

Vasudeva, R. Sahai (1935). *Ind. Jour. Agric. Sci.* **5**, 496-512.

Martin, Hubert (1936) ' The Scientific Principles of Plant Protection ' pp. 50-53.

AWNEDNESS AND ITS INHERITANCE IN RICE

BY

R. L. SETHI, M.Sc., B.Sc. (Edin.), I.A.S.
Officer on Special Duty, Imperial Council of Agricultural Research, India

B. L. SETHI, M.Sc., Ph.D. (Wales)
Officiating Economic Botanist (Sugarcane and Paddy) to Government, United Provinces

AND

T. R. MEHTA, B.Sc.
Officiating Assistant Paddy Specialist, Rice Research Station, Nagina

(Received for publication on 24th November 1936)

(With Plate LI)

CONTENTS

		Pages.
I.	Introduction	589
II.	Review of work on awns	590
III.	Influence of environment	591
IV.	Study of inheritance	593
V.	Inheritance of colour	596
VI.	Summary	597
	Acknowledgment	597
	References	597
	Appendix	598

I. Introduction

The value of awns in cereal crops has been considered dubious by many workers. The rice crop has by no means been an exception. While awnedness of a variety is undesirable from a miller's point of view it is definitely desired by some growers because awns protect the crop, to a considerable extent, from the depredations of birds. This character is of importance to the systematist also who designs to classify his varieties on the basis of this character. For these reasons awns have been a subject of study by several workers. In this paper it is proposed to present a brief review of the knowledge so far gained as well as the results of the study of this character in relation to environment and its inheritance. The study was pursued at the Rice Research Station, Nagina, in the years 1932-36.

II. REVIEW OF WORK ON AWNS

We have no evidence to show that awnedness in rice has the same significant relation to yield as has been described by certain workers in the case of other cereals [Clark, 1924 ; Harlan and Anthony, 1920 ; Hayes, 1923 ; Perlitius [Clark, 1924] and Schmid [Clark, 1924]]. These workers show that length of awns is positively correlated with yield. The statement of Kikwa [1912] that most of the rice varieties in advanced centres of rice cultivation are awnless is one which minimises the importance of awnedness to yield in the case of rice. In the United Provinces, in certain localities, however, the awned varieties are preferred to the awnless. Thus in the *tarai* region (a belt at the foot of the Himalaya mountains) the awned varieties offer resistance to pigs and other wild animals which abound there, and are more commonly grown.

The extreme variability, and therefore the unsuitability of awns in any scheme of classification, has been emphasized by different workers. Thus Graham [1913] says that the length of awns varies within the same variety and is by no means constant. Sethi and Saxena [1930] state about the rices of the United Provinces " No feature is more variable among the rices grown in these provinces than the awn character ". They further state " The length (of awns) varies in different varieties and even within the same variety.... Ordinarily it varies from a tiny little bristle to a maximum of three inches. In many cases awns appear only on a few spikelets near the top of the ear-head, the basal spikelets remaining awnless ". Jones [1927] has pointed out that environment plays an important part in the expression of awnedness, and that some awnless varieties show awns on their secondary tillers. Alam [1931], on the other hand, appears to minimise the influence of environment and to be inclined to use awns as a basis of classification. He says " The more remarkable variation in awn character is not due to environment but due to heredity ". He seems to ignore the fact that even the less remarkable variations in a character throw doubts on the genetic stability or otherwise of a variety when the time comes for judging its homozygosity. Then it is often impossible to say how much variation may or may not be ascribed to heredity, and in that event awnedness, as a basis of classification, is to be deprecated.

On the inheritance of awns not so much work has been done on rice as has been done on wheat. Van der Stok [1910] was one of the first to study the inheritance in rice. He found that awnedness may be either dominant or recessive. In one cross between unawned and long-awned varieties he found F_1 to be longer awned. In the F_2 there was no Mendelian segregation. The awned and the awnless plants were 285 and 71 in numbers and seemed to approach a mono-factorial scheme. In another cross between weakly-awned and awnless varieties the F_1 was awnless and the F_2 showed great variation. The number of constant F_2 plants was much smaller than expected. The parental types were found to segregate while some of the intermediate types bred true. Okada [Ikeno, 1927] found awnedness dominant and obtained a mono-factorial segregation in F_2. Hoshino [Ikeno, 1927] found awning dominant. Kato [Ikeno, 1927] found awned and awnless plants in an F_2 in the numbers 1636 and 540, which represent a 3 : 1 ratio. In other cases the inheritance was less simple and no definite conclusions could be arrived at. Ikeno [1927] obtained results which could not be completely explained. However, a dihybrid mode of inheritance was indicated. Jones [1927] found awnedness partially dominant to awnlessness in F_1. Of the two crosses studied by him one indicated that two genetic factors were involved in the inheritance of awnedness while the other cross indicated only a single factor. Chao [1928] found two factors, one of which exerted greater influence than the other. Alam [1931] got a 15 : 1 ratio of awned and awnless plants in a natural cross.

III. Influence of environment

The notoriety with which awns show environmental variation in rice makes it desirable for us to know the extent to which awn can vary due to environment. This is important, not only in judging the ' purity ' of a crop, but also in arriving at any definite conclusions as regards inheritance of awnedness. One of the environmental factors which is often ignored is the spacing given to the plants. An investigation of this question has been made by Anandan [1933] in Madras and it was shown that crowding of plants resulted in greater awn development. A similar experiment was laid down at the Rice Research Station, Nagina, in 1934 and 1935 to test this effect.

One of the late-maturing types, T 23, was used for the experiment. This type usually possesses awns 0·5 in.-1·0 in. in size on the top few spikelets of the ear, the lower spikelets being free from awns. The seedlings were transplanted in small beds, 5 ft. × 5 ft. in size, and the seedlings given different spacings in the different beds. The spacings given were : 12 in., 9 in., 6 in., 3 in., crowded (*i.e.*, less than 1 in. between seedlings) and ' bunches ' (twelve to twenty seedlings in each hole, the holes spaced 12 in.). The planting was done in two sets of six beds each, one set being heavily manured with Nicifos II to supply 200 lbs. nitrogen per acre, the other set being left unmanured. The manure was given to see if greater awn development in close spacing was due to deficient nutrition.

There was no effect of manure on the development of awns, similar to Anandan's findings, but the effect of spacing was marked, smaller spacing resulting in greater awn development. The differences observed between 12 in., 9 in. and 6 in. spacings were not discernible, nor indeed definite, in 1934, but that between 12 in. and 3 in. or ' crowded ' was very marked. The ' bunches ' behaved, more or less, like widely spaced single plants, though showing more variation from tiller to tiller. In 1935 the differences between 12 in., 6 in., 3 in. and ' crowded ' were clear and measurable but not that between 12 in. and 9 in. In each year fifty ears were harvested at random from each of these treatments. The percentage of awned spikelets (*i.e.*, spikelets with awns 2 mm. or longer) on each ear was counted by separating the awned and the awnless spikelets. Of the former about one-third were taken at random for measurement of awn length in order to get an estimate of the average size of awn in each ear. The averages are presented in Tables I and II.

TABLE I

Effect of different spacings on the development of awns in T 23 in 1934

Spacing	Percentage of awned spikelets	Average length of awns
		(mm.)
12 in. apart each way	32·0	4·83
'Crowded'	90·8	8·18

TABLE II

Effect of different spacings on the development of awns in T 23 in 1935

Spacing	Percentage of awned spikelets	Average length of awns
		(mm.)
12 in. apart each way	35·0	6·65
6 in. apart each way	50·7	8·04
3 in. apart each way	63·1	9·69
'Crowded'	87·5	8·37

Thus closer crowding has resulted in greater awn development. The figures in the above tables are averages of fifty ears. The differences noticed by the eye appeared greater than those suggested by the above figures, awnedness appearing specially prominent in the 'crowded' treatment in which the crop was relatively poor and the awns appeared relatively very prominent on account of the small size of the ears.

The implication of the fact may be this. On a normal field a rice variety possessing awns is likely to show variation in awn development because of the difference in spacing between plants in the fields, thus giving an appearance of impurity even to a pure crop.

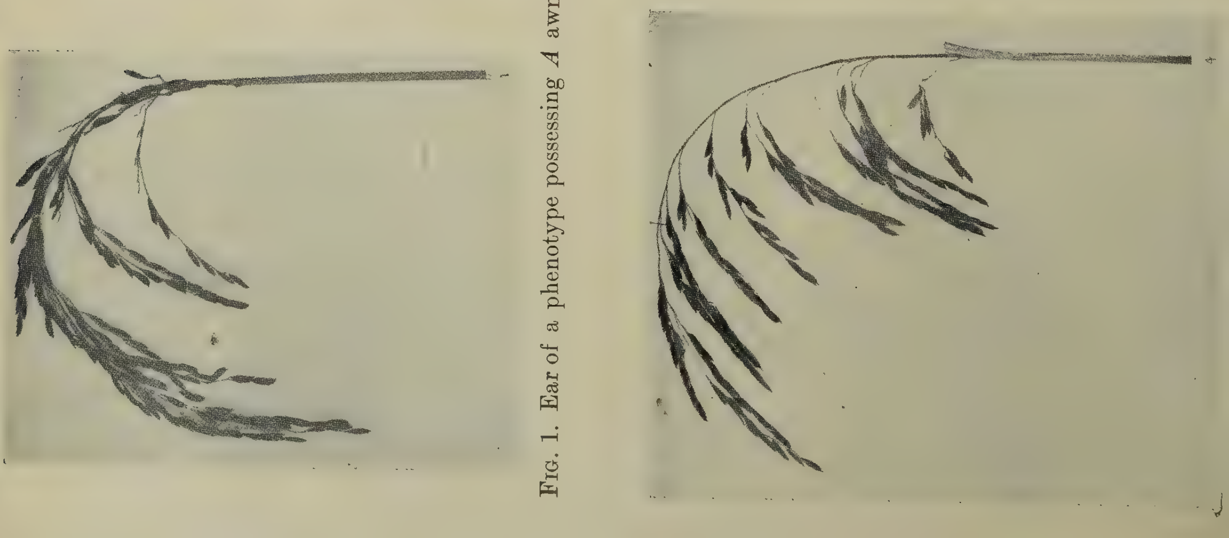


FIG. 1. Ear of a phenotype possessing *A* awns.

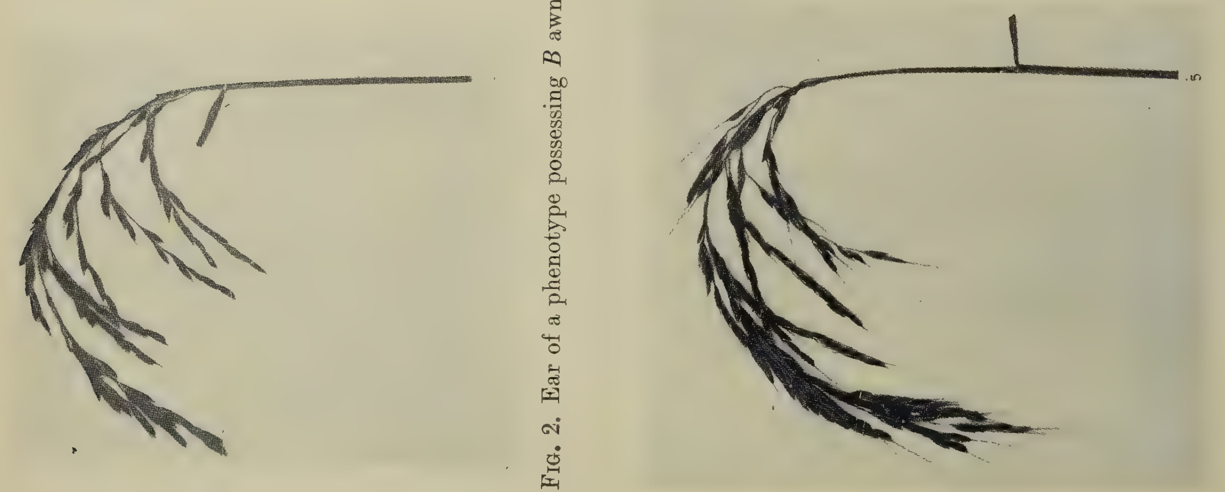


FIG. 2. Ear of a phenotype possessing *B* awns.

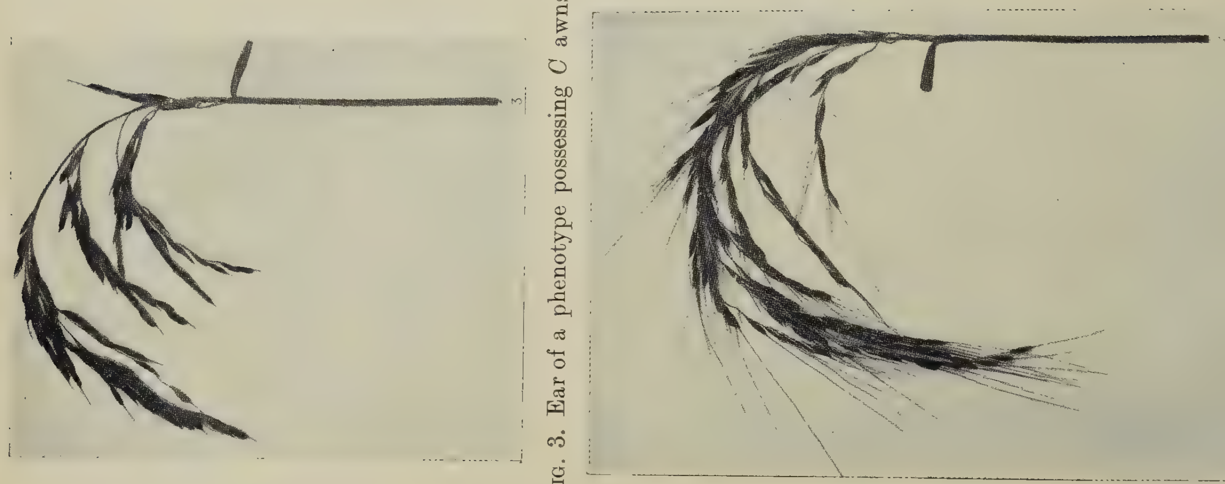


FIG. 3. Ear of a phenotype possessing *C* awns.

IV. STUDY OF INHERITANCE

In the year 1935 an F_2 generation was studied for the inheritance of awnedness. The parents were T 29 and T 30, two of the genetically pure strains grown at Nagina every year. The F_1 was obtained as a result of natural crossing between the two types. T 29 is awnless. T 30 has awns averaging 3-4 cm. on the upper part of the panicle ; the spikelets on the lower part bear much smaller awns, varying from tiny points to 2 cm., while some of the spikelets are completely awnless (Plate LI, fig. 5). The longest awn hardly ever exceeds 4·5 cm. The F_1 had awns only on a few spikelets near the tip of the panicle, the length of awns not exceeding 2 cm. In F_2 all degrees of awning, from that of T 29 to that of T 30, were observed.

We shall first describe the phenotypic classes in which the F_2 was classified for the study of inheritance and represent these classes by symbols for ease of presentation in the subsequent paragraphs. The two parental classes, *viz.* awnless and awned, have been called, respectively 'not awned' (Plate LI, fig. 1) and 'medium awned' (Plate LI, fig. 5), and for convenience will henceforth be denoted as *A* and *E*. The F_1 was called 'tip-awned' or *C* as we shall call it (Plate LI, fig. 3). In F_2 besides the three classes *A*, *E* and *C* were found classes which we shall call *B* (*i.e.*, one or two apical spikelets awnletted (Plate LI, fig. 2) the panicle being otherwise awnless) and *D* (*i.e.*, short-awned, a class lying between *C* and *E*) (Plate LI, fig. 4). The awnedness was expressed both by the length of the awns as well as their frequency. Thus the class *E* had not only longer awns than *D*, it had also a greater number of spikelets provided with awns. Moreover, the length and the frequency decreased in a panicle from the tip to the base.

Were it possible to completely represent the variation in awning only by these classes the study of F_2 and F_3 generations would not have offered much difficulty. As it was, the situation was more complex. Some plants were difficult to classify. Thus we had plants which, when considered on the merit of awn-length, would be placed as *E* but which, when considered on the merit of frequency of awns, would as easily fall into the class *D*. Besides, the character varied from tiller to tiller in certain cases, some tillers leading to an impression of class *C* while one or two possessed *D* awns. Moreover, in some cases, it was difficult to determine whether a spikelet was awned or awnless, because there was a merest trace of awn visible. Such plants could neither be placed conveniently in the *A* nor in the *B* class. The *B* class itself was remarkable in the fact that whereas some of the panicles on a plant were *A* awned others had just one or two spikelets with a short tip.

Classifying the F_2 in the above five classes we came to the following frequencies :—

A	*B*	*C*	*D*	*E*	Total
60	48	47	52	100	307

Actually the number of classes made during the course of observation was larger doubtful cases being classified separately. The figures could, however, not be arranged in the form of definite Mendelian ratios in whatever way the doubtful classes were combined with the other classes. The parental classes were recovered in abundance, besides minor groups of intermediate classes. Seeing that the character under study is a very variable one and that the segregation in F_2 is involved, it is apparent that the classification of the F_2 is not possible on eye judgment. The breeding behaviour of all the F_2 plants would alone definitely point out the various genotypes. For the purpose of study sixty-two F_3 families were raised in 1936. They represented the different classes as follows :

A	*B*	*C*	*D*	*E*	Total
12	12	8	8	22	62

In F_3 all the phenotypes were found to segregate. Moreover, each of the different phenotypes did not show a distinct mode of segregation. Thus many F_2 plants of class *A* behaved similarly to plants of class *B* or of class *C*, each of these segregating into three or more classes, *viz.* *A*, *B*, *C* and *D*. There was a strong tendency in each family to represent the parental class. Thus an *A* F_2 plant gave in F_3 a majority of *A* plants and very few *B*, *C* and *D* plants. Similarly a *B* F_2 plant gave more *B* plants in F_3 than was given by an *A* F_2, if it did not indeed give a majority of *B* plants. The *C* F_2 plants gave all the five classes in F_3. The tendency for the F_2 parental phenotypes to be represented strongly in the corresponding F_3 families has been shown in Table III. In this table the phenotypic frequencies of the F_3 families coming from identical F_2 phenotypic plants have been grouped together. The frequencies are represented as averages of percentage values shown in Appendix I. For details this appendix may be consulted.

TABLE III

Frequencies of awn length of F_3 families grouped according to F_2 parental classes

F_2 phenotypes	Segregation in F_3					Total
	A	*B*	*C*	*D*	*E*	
A	86·9	5·0	6·5	1·6	..	100·0
B	47·1	26·9	16·8	8·1	1·1	100·0
C	23·4	6·0	49·5	16·6	4·5	100·0
D	15·1	9·9	23·1	28·5	23·4	100·0
E	5·7	4·3	12·3	19·1	58·6	100·0

Thus an increase in awnedness of F_2 plants found an expression in the F_3 generation.

The above behaviour of the character in the F_3 generation suggests a quantitative mode of inheritance. Of the different families only two bred true, one from the *A* class and the other from the *E* class (families 53 and 72, Appendix I). That is, out of the twelve F_2 plants of *A* class only one was homozygous. Similarly, out of twenty-two F_2 plants with *E* awns one was homozygous. This works out to five homozygous *A* and five homozygous *E* plants if the total population is considered. This would be the number of homozygous plants we would obtain from the two extreme (*i.e.*, the parental) classes if we had three pairs of factors operating cumulatively. For, in a trihybrid quantitative scheme the F_2 classes representing 6, 5, 4, 3, 2, 1 and 0 genes would be represented in the proportion of 1 : 6 : 15 : 20 : 15 : 6 : 1. That is, a total of sixty-four plants would give only one plant with all the six genes and one plant with no gene, and these would breed pure. We can assume that the true breeding *E* awned and *A* awned families obtained in our experiment were, respectively, from plants possessing six and no genes. In a population of 307 F_2 plants the theoretical number of such plants works out at 4·8 which is very near the 5 observed in our experiment. More than this it is not possible to say, because the ratios observed in the different families were involved and were not amenable to solution.

A similar situation prevailed in another cross between T 15 and T 34, the former having *C* awns and the latter *E* awns. In the F_3 generation, grown in 1932, families were obtained which showed wide segregations, some even transgressing the parental limits. Thus although both the parents were awned many of the F_3 segregates were awnless. Selections were made from an F_3 family which segregated widely giving the following frequencies :—

A, 12 ; *C*, 19 ; *D*, 37 ; *E*, 44 and *F*, 5.

(*F* represents long awns, and is a class above *E*) (Plate LI, fig. 6).

Progenies of forty-three of these F_3 plants were studied in 1933. Every one of the progenies segregated into two or more classes, indicating that the genic difference between the two parents was greater still in this cross than in that between T 29 and T 30. Otherwise remarks similar to those for the latter cross apply to this cross as well. A summary of the forty-three F_4 families of this cross is given in Appendix II. Selections made in the F_4, F_5 and F_6 generations continued to give segregating progenies.

V. INHERITANCE OF COLOUR

An F_2 generation of a cross between T 1 and T 131 was grown in 1931, and the awn was found to segregate in two colour classes, *viz.* 'purple' and 'no colour'. The frequencies were 158 'purples' and 50 'no colours', indicating a simple 3 : 1 ratio. In the F_3 generation true breeding families of 'purple' as well as the 'no colour' classes were secured. Seven segregating families were examined and the frequencies of the two classes were found to be as given in Table IV.

TABLE IV

Segregation of awn colour in F_3 families of Cross, T 1 × T 131

Family No.	Segregation		Total
	Purple	No colour	
X18/1EN 17	84	28	112
X18/1EN 20	89	18	107
X18/1EN 35	58	22	80
X18/1EN 39	112	32	144
X18/1EN 100	57	15	72
X18/1EN 104	62	33	95
X18/1EN 181	86	20	106
Total	548	168	716

Evidently the colour segregated into a 3 : 1 ratio in each family. The calculated total frequencies for the two classes are, respectively, 537 and 179, which represent a deviation of 11 from the observed value. The standard error is 11·6 which shows that the deviation is insignificant, and can be ascribed to chance.

VI. SUMMARY

Awnedness in rice and its inheritance have been discussed. The variability of the awn and the influence of environment on its development have been presented to show that crowding of plants tends to increase awn development. This is perhaps not due to deficient nutrition because even when adequate nutrition was supplied by heavy manuring the relation between the crowding and awnedness remained unaltered. In inheritance the length of awns was found to be governed by three or more genes acting cumulatively. The colour of awn was due to a simple gene.

ACKNOWLEDGMENT

This work was carried out as part of the Rice Research Scheme, Nagina, financed by the Imperial Council of Agricultural Research, India, and thanks are due to the Council for providing the necessary funds. Thanks are also due to Mr. R. K. Tandon, M.Sc., one of the Research Assistants, who was of great help in collecting part of the data presented in this paper.

REFERENCES

Alam, M. (1931). *Agric. & Live-stock in Ind.* **1**, 497-511.

Anandan, M. (1933). *Current Sci.* **2**, 8, 284-285.

Chao, L. F. (1928). *Genetics* **13**, 2, 133-169.

Clark, J. A. (1924). *Jour. Agric. Res.* **29**, 1-47.

Graham, R. J. D. (1913). *Mem. Dept. Agric. Ind.* (*Bot. Ser.*) **6**, 209.

Harlan, H. V. and Anthony, S. (1920). *Jour. Agric. Res.* **19**, 431-472.

Hayes, H. K. (1923). *Minnesota Stud. Plant Sci.* **4**, 163-183.

Ikeno, S. (1927). *Bibl. Genet.* **3**, 245-310.

Jones, J. W. (1927). *Jour. Amer. Soc. Agron.* **19**, 830-839.

Kato : Quoted from Ikeno, S. (1927).

Kikwa, S. (1912). *Jour. Col. Agric. Imp. Univ.* (*Tokyo*) **3**, 2.

Okada: Quoted from Ikeno, S. (1927).

Perlitius, L. : Quoted from Clark, J. A. (1924).

Schmid, B. (1898) : Quoted from Clark, J. A. (1924).

Sethi, R. L. and Saxena, B. P. (1930). *Mem. Dept. Agric. Ind.* (*Bot. Ser.*) **18**, 6, 149-209.

Van der Stok : Quoted from Ikeno, S. (1927).

APPENDIX I

Frequencies of awn classes in F_3 T 29 × T 30 shown as percentages of the total populations

Family	F_2 character	Segregation in F_3					Total
		A	*B*	*C*	*D*	*E*	
17	*A*	96·6	1·7	1·7	..	..	100·0
21	,,	91·7	8·3	..	..	..	100·0
27	,,	68·4	5·3	21·1	5·3	..	100·1
32	,,	81·4	13·6	5·1	..	..	100·1
48	,,	71·9	5·3	15·9	7·0	..	100·1
51	,,	95·2	3·2	1·6	..	..	100·0
53	,,	100·0	..	..	..	..	100·0
57	,,	94·6	1·8	3·6	..	..	100·0
59	,,	85·9	6·3	7·8	..	..	100·0
68	,,	84·4	7·8	6·3	1·6	..	100·1
74	,,	83·7	6·1	6·1	4·1	..	100·0
76	,,	88·7	..	9·4	1·9	..	100·0
Average		86·9	5·0	6·5	1·6	0·0	100·0
22	B	38·3	8·3	36·7	15·0	1·7	100·0
34	,,	67·8	18·6	6·8	6·8	..	100·0
35	,,	29·3	53·5	10·3	6·9	..	100·0
38	,,	61·9	11·1	19·0	6·3	1·6	99·9
39	,,	11·3	69·4	14·5	4·8	..	100·0
47	,,	37·3	15·3	22·0	23·7	1·7	100·0
58	,,	70·0	18·3	11·7	..	..	100·0
60	,,	61·3	6·5	22·6	9·7	..	100·1
61	,,	3·6	54·5	27·3	14·5	..	99·9
71	,,	72·5	27·5	..	..	..	100·0
77	,,	30·2	20·9	30·2	9·3	9·3	99·9
78	,,	81·4	18·6	..	..	..	100·0
Average		47·1	26·9	16·8	8·1	1·1	100·0
19	*C*	55·7	1·6	29·5	8·2	4·9	99·9
37	,,	1·7	..	72·4	25·9	..	100·0
44	,,	28·1	7·0	28·1	28·1	8·7	100·0
45	,,	9·7	11·3	79·0	..	..	100·0
49	,,	27·9	4·9	49·2	18·0	..	100·0
67	,,	31·5	11·1	24·1	25·9	7·4	100·0
69	,,	16·0	10·0	70·0	4·0	..	100·0
80	,,	16·7	2·1	43·8	22·9	14·6	100·1
Average		23·4	6·0	49·5	16·6	4·5	100·0

APPENDIX I—*contd.*

F_3 family	F_2 character	Segregation in F_3					Total
		A	*B*	*C*	*D*	*E*	
26	D	6·7	15·0	30·0	35·0	13·3	100·0
30	,,	3·2	17·7	35·5	24·2	19·4	100·0
31	,,	27·8	9·6	14·8	16·6	31·4	100·2
33	,,	11·7	6·7	18·3	28·3	35·0	100·0
43	,,	15·8	15·8	14·0	43·9	10·6	100·1
46	,,	12·9	8·1	29·0	22·6	27·4	100·0
52	,,	23·3	5·0	31·7	35·0	5·0	100·0
73	,,	19·4	1·6	11·3	22·6	45·2	100·1
Average .		15·1	9·9	23·1	28·5	23·4	100·0
15	*E*	..	1·9	18·5	27·8	51·8	100·0
16	,,	5·5	10·9	20·0	25·5	38·2	100·1
18	,,	38·3	10·0	16·7	15·0	20·0	100·0
20	,,	..	..	1·8	25·5	72·7	100·0
23	,,	..	..	..	6·9	93·1	100·0
24	,,	..	..	..	20·0	80·0	100·0
25	,,	1·7	5·1	20·3	18·6	54·2	99·9
28	,,	1·8	3·5	24·6	26·3	43·8	100·0
29	,,	..	10·5	15·8	22·8	50·9	100·0
36	,,	28·1	8·7	14·0	29·8	19·3	99·9
40	,,	6·9	17·2	13·8	20·7	41·4	100·0
55	,,	1·8	10·9	14·5	16·4	56·4	100·0
56	,,	1·6	1·6	6·5	27·4	62·9	100·0
62	,,	..	2·3	..	7·0	90·7	100·0
63	,,	5·6	3·7	22·2	20·4	48·1	100·0
64	,,	15·0	3·3	20·0	18·5	43·3	100·1
65	,,	..	..	13·2	13·2	73·6	100·0
66	,,	..	..	4·8	29·0	66·1	99·9
70	,,	..	..	17·3	5·8	76·9	100·0
72	,,	..	..	..	..	100·0	100·0
75	,,	19·0	4·8	21·4	11·9	42·9	100·0
79	,,	..	..	5·7	32·1	62·3	100·1
Average .		5·7	4·3	12·3	19·1	58·6	100·0

APPENDIX II

Frequencies of awn classes in F_4—T 15 × T 34, 1933

Family	F_3 character	Segregation in F_4						Total
		A	*B*	*C*	*D*	*E*	*F*	
26—14	*A*	15	31	42	2	..	..	90
26—18	,,	8	17	75	14	..	..	114
26—21	,,	14	13	61	16	4	..	108
26—30	,,	4	7	72	15	8	..	106
26—45	,,	34	18	52	5	..	..	109
26—69	,,	6	5	72	18	9	..	110
26—79	,,	10	24	34	26	4	..	98
26—15	*C*	..	4	44	68	7	..	123
26—26	,,	1	2	17	67	26	..	113
26—31	,,	1	1	27	43	36	..	108
26—35	,,	..	2	14	44	39	1	100
26—40	,,	..	..	17	52	50	..	119
26—46	,,	..	1	32	70	1	..	104
26—76	,,	..	5	42	32	14	..	93
26—77	,,	1	..	60	52	2	..	115
26—4	*D*	..	..	2	54	34	..	90
26—8	,,	..	1	60	43	..	..	104
26—12	,,	..	..	1	28	65	..	94
26—13	,,	..	1	33	51	29	..	114
26—16	,,	..	..	6	54	56	..	116
26—42	,,	..	..	7	91	3	..	101
26—43	,,	..	..	6	71	28	..	105
26—62	,,	3	3	33	38	34	2	113
26—65	,,	2	4	26	55	22	..	109
26—1	*E*	..	..	8	23	17	..	48
26—2	,,	..	3	19	38	28	2	90
26—6	,,	..	..	..	11	82	4	97
26—7	,,	..	1	8	41	60	..	110
26—25	,,	..	..	2	32	51	..	85
26—27	,,	..	..	1	27	57	..	85
26—29	,,	1	5	9	20	53	1	89
26—33	,,	..	..	..	6	110	..	116
26—38	,,	..	..	1	6	103	..	110
26—48	,,	..	..	1	34	66	..	101
26—50	,,	..	..	4	19	80	5	108
26—52	,,	..	..	..	15	97	2	114
26—57	,,	..	..	..	8	111	..	119
26—67	,,	..	..	..	17	80	..	97
26—74	,,	..	..	1	14	83	..	98
26—80	,,	..	..	2	3	100	..	105
26—81	,,	..	..	..	23	81	..	104
26—22	*F*	..	..	..	..	37	20	57
26—47	,,	..	..	..	2	76	6	84

SAMPLING OF SUGARCANE FOR CHEMICAL ANALYSIS, I

BY

RAMJI NARAIN, PH.D., D.Sc.
Second Agricultural Chemist, Punjab, Lyallpur

AND

AZMAT SINGH, L.Ag.
Agricultural Research Institute, Lyallpur

(Received for publication on 22nd August, 1936)

(With four text-figures)

INTRODUCTION

THE Punjab Department of Agriculture is engaged in carrying out at a number of its research stations, a considerable amount of work on the development of sugarcane industry of the Province. In connection with the field trials on the various aspects of the problem of sugarcane production, chemical analysis of a large number of samples is being done every year. In order to understand properly the significance of the results of these analyses, it is essential that the analytical figures for the various constituents should be accurately determined from representative samples carefully obtained from a plot or field of sugarcane. It has been recognised by different workers that in dealing with a highly heterogeneous crop like sugarcane, it is extremely difficult to obtain a single sample of convenient size which will truly represent the condition of the standing crop. This difficulty has been felt not only in the case of the standing crop, but even in sampling cane from the harvested crop received in the factory. According to Moberley [1935] it is universally accepted that cane sampling is barely practicable, nevertheless, it was believed by him that reasonably concordant results might possibly be obtained with samples of about 250 lbs. of cane. In view of these difficulties it has been suggested by some workers that a sample from the entire bulk of juice obtained from all the canes from any plot or field under any particular treatment, harvested and crushed under uniform conditions at one time alone will constitute a reliable single sample. This however, as will be readily understood, can be possible only when the size of the plot is small and consequently the weight of the cane to be crushed not inordinately great and when the crop is to be finally harvested. In the case of large-sized samples, however, as will be shown later, the figures for juice percentage, owing to the lack of uniformity in the conditions under which cane may be crushed, involve a large amount of error. Moreover, in such a case considerable time must lapse before the sample of juice can be obtained for analysis, with the result that the analytical data obtained from juice which has been stored over such a long period will also be subject to some error due to changes in its composition which are likely to occur during this period of storage. It is therefore necessary for the purpose of analysis to obtain a sample of convenient size which may, as far as possible, be representative of the entire population. Clarke and Hadi [1908] working at the Partabgarh Experimental Station, found that when a comparatively small sample of cane is taken from different places in the same field under the same variety, it was not possible to get an accurate estimate of the yield of juice and its composition, the figures obtained differing widely, excepting where several hundred lbs. of juice were employed; and even then considerable variations are likely to occur. For instance they found that the sucrose content varied from 13·22 to 16·19 per cent. As a result of various trials, they decided to sample the entire field in such a way that four strips, one stool wide, were taken at equal distances apart, starting from the right hand and cutting down the whole length of the field. The juice from the four strips was mixed and sampled. The cutting was continued in the same way from right to left, four strips being used for each sample consisting of 500 to 1000 lbs. each. This method gave a better agreement but even here differences of 0·5 per cent in the amount of sucrose contained in the juice were not uncommon. Dymond [1935] also analysed separately all the canes from every sixth line in a field of Uba cane from plots on which no fertilizers had been used. The sucrose percentage on cane varied between 13·34 and 6·96. This surely is a much bigger variation than that which was found by Clarke and Hadi [1908]. Even considering that these latter workers crushed four strips or lines against one by Dymond [1935], the amount of variations obtained in his case is difficult to explain. Leather [1913], working on the same problem, found that it was not necessary to take so large a sample as recommended by these authors. He suggested that a much smaller sample consisting of say 200 canes may be taken in nine or ten lots of three canes each from approximately equidistant points in each of the specified rows in a plot of cane, the number of such points being eight or nine, when the plot is approximately square. When the plot was not square the distribution of the points from which to cut out the cane was made to suit the dimensions. When the sample was taken in this manner, the error in sampling was not greater than when the sample was taken according to the Clarke and Hadi method described above. Davis [1934] from his experience gained at Peurto Rico Agricultural Experimental Station, recommends another method, according to which two men work on opposite sides of the field. Each man punches a core from the centre of each of 100 canes. The 200 cores are placed together in a strong fruit press, and a composite juice sample is extracted. It will be seen, however, that the method can be of use only in getting qualitative data regarding different types of canes and can give no idea about the juice percentage. The method of sampling adopted in Queensland [1933], which is different from the above methods, consists in picking canes of an average degree of purity from a selected number of stools in each acre of the field. It will be evident, however, that the introduction of deliberate selection twice in the sampling will result in increasing the error through personal bias, which will not be the same for different individuals or even for the same individual at different times. The selected average canes from different stools may or may not be alike, and, for this reason, the average condition of the crop, as represented by the sample, will very likely be different from the true average of total crop. As has been pointed out by Kobus [1906], very great variations occur amongst the individual canes composing a stool. To select from these a cane of average composition is not an easy task.

In order to obtain a sample of cane representative of the entire plot or field, the practice followed in these laboratories has been to take a number of stools at random from points scattered over different places in the field and then combine all the canes, in these stools, to form one sample. In other words, the stool has been the unit rather than a single cane. The maximum difference in sucrose percentage amongst canes with the lowest and highest sucrose content as given by Leather [1913] was 14·2, 14·9, and 17·2, in the case of China, Mungo, and Khari varieties respectively, as against 2·1, 2·9 and 2·5 obtained by us for Katha, Co 285, and Co 312 respectively in the case of one-stool sample (Table II). Thus by taking a stool as the unit for sampling we eliminate a good deal of variation due to differences in the individual canes resulting from differential maturity ; and since the immature canes are as much a part of the total population as the so-called canes of average purity, there seems to be no justification in fixing this limitation in obtaining a representative sample. A random sample composed of a number of stools obtained from various places in the field without following any regular order is more likely to yield a sample which will be better representative.

In view of the considerable amount of work which is in progress in the province in connection with sugarcane survey and other departmental investigations connected with this crop, about forty samples per day are being analysed in these laboratories. It can be easily realized that the collection of so many samples on the lines suggested by Clarke and Hadi [1908] would mean harvesting a very large portion of the crop and it would not be possible to crush so many samples without introducing errors due to great interval in the times of crushing of some of the samples. Taking so many samples by the Leather method would also be very tedious and time-taking. Moreover, when it is desired to study the effects of season and manurial treatments upon the periods of maximum ripeness or the effects of frost, etc., the sugarcane crop has to be analysed at regular intervals with the result that if a large fraction of the crop as mentioned above is used for each analysis, not only will it be difficult to handle such a large sample, but also so much of the crop would be used for analyses only, that enough may not be left to give reliable data concerning the main object of the experiment.

In order to avoid confusion it will be advisable, before proceeding further, to indicate the strictly technical sense in which the following terms have been used in this paper :—

(*a*) 'Units'—the ultimate parts of a sample series which in the case of the present investigation consist of one stool of sugarcane.

(*b*) 'Sample units'—those parts of a sample series which are obtained by combining one or more units picked at random.

(*c*) 'Sample series'—the aggregate of sampling units, representing the population, where n denotes the number of sample units in any series.

OBJECT

In view of what has been said above regarding the impracticability of obtaining samples of cane which may be exactly alike, need was felt of comparing amongst themselves a number of sample units of the same size as well as of different sizes, with the object of determining the standard deviation that sample units of different sizes are subject to and therefrom to get an estimate of the standard error of the samples made up of units of different sizes. It must be recognised that any representative sample of cane, irrespective of its size, will always be subject to a certain amount of error, the extent of which will depend upon the size of the sample, the manner of taking it and the extent of differences amongst the individuals composing it. We have shown that individual differences can be eliminated to a very great extent by taking the stool rather than the cane as the unit of sampling. The present investigation was, therefore, undertaken with the twofold object (*i*) of fixing the limits of accuracy within which the mean value of different constituents of sugarcane from any plot should lie so as to yield reliable information about the condition of the crop as influenced by different treatments, manurial, cultural and such other, and (*ii*) of determining the size and manner of taking the sample which would be subject to an error not greater than the limit of tolerance so selected.

FACTORS INFLUENCING THE RESULT OF ANALYSIS

It is well known that the analytical results relating to sugarcane may be subject to errors arising from a number of sources. In addition to errors in sampling which form the subject matter of the present investigation, two more factors have been recognised as responsible for introducing a certain amount of error in the analytical data, *viz.* (*a*) the extraction of the juice and (*b*) the methods of analyses. A brief discussion of the part played by each in influencing the results of analysis is given below.

(*a*) *Extraction of juice*

It has been observed at Lyallpur that unless the sugarcane samples are crushed under uniform conditions the figures for juice percentage even from samples of the same variety grown under identical conditions in the same field, may give widely different values, which in all cases might not be due to differences in the quality of the samples analysed. It is evident from a cursory glance at Table I, which gives the results of a preliminary crushing test with Co 290 carried out at Lyallpur, that the precentage extraction of juice from cane is greatly influenced by the interaction of three different factors, namely :—

(*i*) Setting of the mill.

(*ii*) Speed of the working rollers.

and (*iii*) Pressure, *i.e.* the number of canes fed at a time.

Table I

Showing the interaction of different factors on the extraction of juice from sugarcane

Serial No.	Description			Weight of canes (oz.)	Weight of juice (oz.)	Juice (per cent)	Remarks
	Setting of the mill	Pressure, *i.e.* No. of canes fed at a time	Speed of working of rollers				
1	Ordinary close	2	Low	331	219	66·2	The speed of the rollers was regulated by the speed of the bullocks driving the mill ; low speed corresponds to an easy pace ; medium to quick and high when the bullocks were running. Ordinarily the canes are crushed at a speed lying midway between low and medium.
2	„	3	„	322	215	66·8	
3	„	5	„	327	210	64·2	
4	„	2	Medium	336	217	64·6	
5	„	3	„	327	211	64·5	
6	„	5	„	339	211	62·2	
7	„	2	High	324	203	62·6	
8	„	3	„	329	206	62·6	
9	„	5	„	334	184	55·1	
10	Extra close	2	Low	325	220	67·7	
11	„	3	„	326	219	67·2	
12	„	5	„	328	192	58·5	
13	„	2	Medium	334	222	66·5	
14	„	3	„	331	215	64·9	
15	„	5	„	335	196	58·5	
16	„	2	High	334	215	64·4	
17	„	3	„	340	209	61·5	
18	„	5	„	329	185	56·2	

For the purpose of this investigation as uniform samples of canes as possible were selected from one stack of stripped canes and crushed in the mill under eighteen different conditions as per details given in the table. It is recognised that the data presented are not sufficient,* and therefore on the basis of these alone, it is not proposed to enter into a detailed discussion of the effects of the mutual interaction of different factors present during crushing. It will be seen however, that there is a clear indication that, if results of any value are to be obtained, crushing should be conducted under strictly controlled and uniform conditions. The highest percentage of extraction was obtained when the mill was set up very close, the pressure on the rollers was regulated by feeding two to three canes at a time and the bullocks working the mill were moving at an easy pace. For the present investigation the conditions under which crushing was carried out were therefore kept as strictly uniform as possible and it is reasonable to conclude that differences in the juice percentage between different samples of the same variety are due more to the differences in the plant rather than in the crushing conditions. Even with all the above precautions, however, a small amount of error due to milling is likely to persist.

(*b*) *Analysis of the juice*

Figures obtained by analysing duplicate samples from the same bulk of juice show that the chemical and optical methods used for the analysis of juice have now reached such a degree of perfection that, excluding a small personal error, these may well be taken to give identical results for similar samples and to represent the true composition of the juice. For routine analysis therefore these may be regarded as free from errors, and variations in the results of chemical analysis of replicate samples from the same plot may well be taken as a measure of the extent of errors in sampling.

SAMPLING

It may be concluded, therefore, that differences in the analyses of replicate sample units of the same variety are mainly due to differences in the samples themselves and their extent could be reduced only if we could improve the sampling technique. The investigation, therefore, resolved itself into a study of the comparison of analytical figures obtained by analysing replicate sample units of various sizes. As expected, the accuracy of the mean value for any constituent of sugarcane will depend directly upon the size of the sample. For the sake of comparison of data obtained on a uniform basis it was considered advisable therefore to provide in each sample series a sufficiently large number of sample units, made up of the same number of units as far as any one particular sample series is concerned.

---

*The effect of different factors in influencing the percentage extraction of juice from different varieties of cane will be examined in greater detail in the coming cane season.

The investigation was carried out at two different places, *i.e.* Montgomery and Lyallpur. At the former place three varieties of sugarcane, *viz.* Katha (a local thin cane), Co 285 (medium cane) and Co 312 (thick cane) were selected for this study, and it was decided to obtain in the first instance sixteen units of one stool each from plots (1/10th of an acre in size) under each variety. A slight departure was, however, made in the case of the Katha variety, where two stools constituted the unit because one stool of this cane did not yield enough juice for analysis. But for the sake of uniformity of description the unit in this case has also been referred to as one stool. From these initial sixteen one-stool units for each variety increasingly bigger sample units consisting of two, three and four stools respectively were constructed by picking at random from the original series of sixteen units, the required number of units necessary for making the composite sample units and then calculating the percentage figures for the composite sample units by adding together the absolute amounts of the weights of canes, juice, total solids and sucrose contained in each of the initial one-stool unit constituting the composite sample unit. The units so withdrawn were in all cases replaced in the original series before this was used for any further random selection. Table II gives the results of analysis of the original sixteen samples each consisting of one stool. In this way four sample series each consisting of sixteen sample units made up of one, two, three, and four stool units, were obtained. As will be seen later, the results for the Montgomery varieties show that error decreases with increase in the size of the sample units. Therefore at Lyallpur it was decided to pick up from the field three stools at random and consider them to collectively constitute the unit as against a one-stool unit at Montgomery. The variety selected for investigation here was Co 318 and forty-eight units each consisting of three stools, picked at random from all over the plot (=1/7th of an acre in size) were taken for the initial sample series and analysed separately for juice percentage and other constituents of the juice. Just as in the case of the Montgomery samples, different sample series comprising sample units of increasingly bigger sizes—6, 9, 12, 18, 24, 30, 36 stools—were constructed by picking at random, from the initial forty-eight three-stool units, the required number of individuals and combining them to give sample units of the desired size. In this manner eight sample series each consisting of forty-eight sample units were constructed, and it will be recognised that this is a fairly large number for arriving at an accurate estimate of the standard deviation of the individual or the standard error of the mean value. The results of the chemical analysis of these forty-eight initial units obtained at Lyallpur are given in Table III.

TABLE II

Showing the data and analysis of the original sixteen one-stool samples of each of the Katha, Co 285 and Co 312 varieties of sugarcane from Montgomery

Sample No.	Katha					Co 285					Co 312				
	Weight of canes (oz.)	Weight of juice (oz.)	Juice per cent	Percentage on juice		Weight of canes (oz.)	Weight of juice (oz.)	Juice per cent	Percentage on juice		Weight of canes (oz.)	Weight juice (oz.)	Juice per cent	Percentage on juice	
				Total solids	Sucrose				Total solids	Sucrose				Total solids	Sucrose
1	55	34	61·8	20·4	17·4	62	44	71·0	17·2	13·1	91	59	64·8	16·9	14·3
2	51	32	62·7	19·5	15·9	62	44	71·0	16·2	12·0	101	69	68·3	16·7	14·0
3	48	28	58·3	20·5	17·5	63	42	66·7	16·0	11·4	93	65	69·9	16·3	13·5
4	45	26	57·8	20·4	17·4	62	44	71·0	16·2	11·7	89	62	69·7	16·9	14·3
5	45	26	57·8	20·6	17·5	69	48	69·6	16·4	12·0	94	60	63·8	18·3	15·6
6	47	28	59·6	20·8	17·6	62	43	69·4	16·6	12·6	74	50	67·6	17·5	14·5
7	44	27	61·4	21·6	17·9	62	43	69·3	15·7	10·9	89	63	70·8	18·2	15·4
8	48	26	54·2	20·6	16·8	63	45	71·4	17·5	13·3	80	55	68·8	16·9	14·4
9	49	30	61·2	21·1	17·9	73	49	67·1	17·8	13·8	81	55	67·9	18·0	15·3
10	46	26	56·5	20·9	17·4	72	49	68·1	16·5	12·3	79	54	68·4	18·3	15·7
11	46	27	58·7	20·5	17·3	71	50	70·4	16·7	12·5	108	72	66·7	18·5	16·0
12	52	30	57·7	21·3	17·5	68	48	70·6	16·1	11·6	110	72	65·5	17·5	14·7
13	50	30	60·0	20·9	18·0	69	47	68·1	15·9	11·4	94	63	67·0	18·2	15·5
14	51	33	64·7	20·3	17·5	71	49	69·0	17·5	13·2	75	51	68·0	17·6	15·1
15	51	29	56·9	21·2	17·6	70	49	70·0	16·2	12·0	105	73	69·5	18·0	15·5
16	51	33	64·7	19·9	16·1	74	51	68·9	17·1	12·8	101	69	68·3	16·9	14·2

TABLE III

Showing the data and analyses of the original forty-eight three-stool samples of the Co 318 from Lyallpur

Sample No.	Weight of canes (oz.)	Weight of juice (oz.)	Juice per cent	Percentage on juice			Sample No.	Weight of canes (oz.)	Weight of juice (oz.)	Juice per cent	Percentage on juice			Sample No.	Weight of canes (oz.)	Weight of juice (oz·)	Juice per cent	Percentage on juice		
				Total solids	Suc-rose	Glu-cose					Total solids	Suc-rose	Glu-cose					Total solids	Suc-rose	Glu-cose
1	178	109	61·2	20·6	15·8	0·25	17	164	103	62·8	20·2	15·6	0·26	33	124	82	66·1	20·0	15·4	0·42
2	128	79	61·6	20·2	15·7	0·21	18	153	95	62·1	19·3	14·4	0·50	34	114	69	60·5	19·5	14·9	0·38
3	115	65	56·5	19·2	14·1	0·36	19	155	97	62·5	20·0	15·1	0·37	35	169	101	59·6	20·0	15·2	0·41
4	152	97	63·4	19·4	14·4	0·48	20	169	104	61·5	19·8	15·3	0·29	36	104	65	62·5	19·5	14·6	0·50
5	158	102	64·5	20·2	15·6	0·34	21	220	133	60·5	20·1	15·5	0·29	37	116	70	60·4	20·0	15·7	0·32
6	151	96	63·6	19·8	15·1	0·26	22	123	78	63·5	20·5	15·7	0·29	38	163	100	61·3	20·1	15·5	0·42
7	145	93	64·2	20·1	15·5	0·28	23	119	73	61·5	19·0	14·0	0·41	39	212	131	61·9	20·1	16·1	0·32
8	117	60	65·0	20·2	15·4	0·35	24	96	59	61·5	19·9	15·2	0·45	40	125	78	62·4	20·5	16·0	0·29
9	196	135	69·0	20·4	15·8	0·28	25	165	105	63·5	19·9	15·3	0·43	41	148	102	69·0	19·1	14·7	0·49
10	118	68	57·6	19·0	14·5	0·39	26	114	71	62·3	20·0	15·4	0·37	42	177	110	62·2	20·2	15·9	0·27
11	109	68	62·3	20·0	15·6	0·27	27	105	64	61·0	20·6	16·0	0·35	43	126	77	61·1	20·2	15·5	0·38
12	129	84	65·0	20·1	15·3	0·35	28	179	113	63·1	20·1	15·7	0·33	44	120	74	61·6	20·1	15·5	0·45
13	109	67	61·5	20·1	15·1	0·38	29	117	74	63·3	20·4	16·0	0·31	45	115	71	61·7	20·8	16·0	0·41
14	198	124	62·7	20·3	15·6	0·36	30	133	83	62·3	18·8	13·9	0·42	46	183	114	62·0	20·6	15·8	0·38
15	133	85	63·9	20·5	15·9	0·25	31	169	104	61·5	20·4	16·0	0·31	47	147	89	60·2	19·3	14·5	0·58
16	143	95	66·5	19·1	14·2	0·54	32	127	80	63·0	20·0	15·6	0·39	48	176	107	60·8	19·5	14·9	0·52

As is clear from the details given above, from the original forty-eight three-stool units, 384 (8 × 48) sample units of the above-mentioned sizes were built up, and the total number of stools so dealt with comes to 6624 (138 × 48). Perhaps it would have been better if all the samples of different sizes had been made up from 6624 individual stools, each picked at random from the entire field. This would, however, have involved a tremendous amount of work in connection with the weighing of each sample unit, crushing it under uniform conditions (any change in which operation affects the figures for juice percentage considerably) and analysing the juice in the laboratory. It may be easily recognised that in crushing the 1st and the 384th sample, considerable time must lapse which in itself would introduce a fairly large amount of error following changes in the composition of the juice in the cane during this period. The operation of crushing all these samples weighing about ten tons would require six bullock-driven crushing mills to finish the work in one full working day of ten hours. Further at the time this investigation was started, no data were available which could have given any indication as to how many different sizes of sample units will have to be considered, in order to obtain the desired information regarding the gain in precision following each increase in the size of the sample unit.

For sucrose which is the most important constituent of sugarcane, the statistical constants $\beta_1 = 0{\cdot}67 \pm 0{\cdot}38$ and $\beta_2 = 2{\cdot}70 \pm 0{\cdot}58$ calculated for the initial forty-eight three-stool units, show that according to criterion for normality [Elderton, 1927] this number represents fairly accurately the normal population of the field.

STATISTICAL EXAMINATION OF THE DATA

The analytical figures for juice, total solids, sucrose and in the case of Co 318 also glucose (the last three expressed as percentages on juice) for sample units of increasingly bigger sizes, constructed on lines already indicated, were examined statistically both for the Montgomery and for the Lyallpur samples. The mean values together with their standard deviations and standard errors and percentage standard errors calculated for these different sample units are given in Tables IV and VIII. The percentage standard error for juice and its other constituents for sample units of various sizes are also shown graphically in Figs. 1-3. Owing to lack of facilities glucose could not be estimated in the Montgomery samples. Further in the case of the Lyallpur samples it was not considered necessary to work out the figures for total solids, sucrose and glucose for sample units composed of more than twenty-four stools, because the limits of error already attained for sample units of this size were found to be sufficiently low for all practical purposes. The details of calculation, which are well-known, are not given.

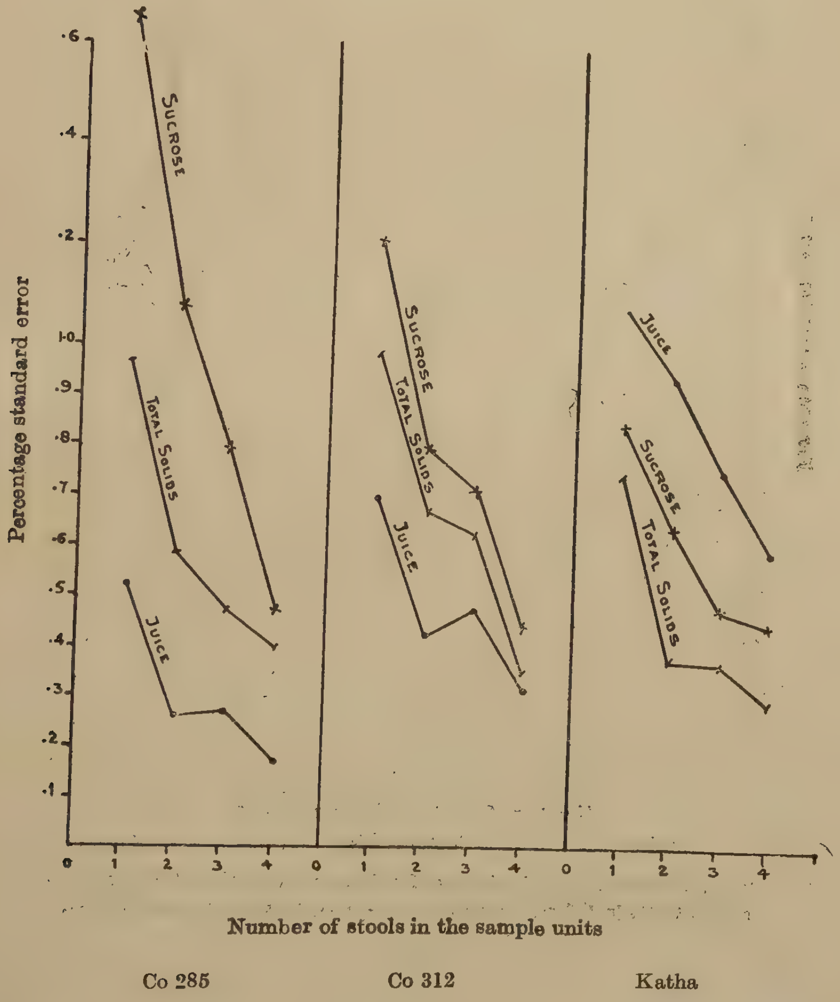


FIG. 1. Percentage standard errors for different constituents of the sugarcane varieties, Co 285, Co 312 and Katha.

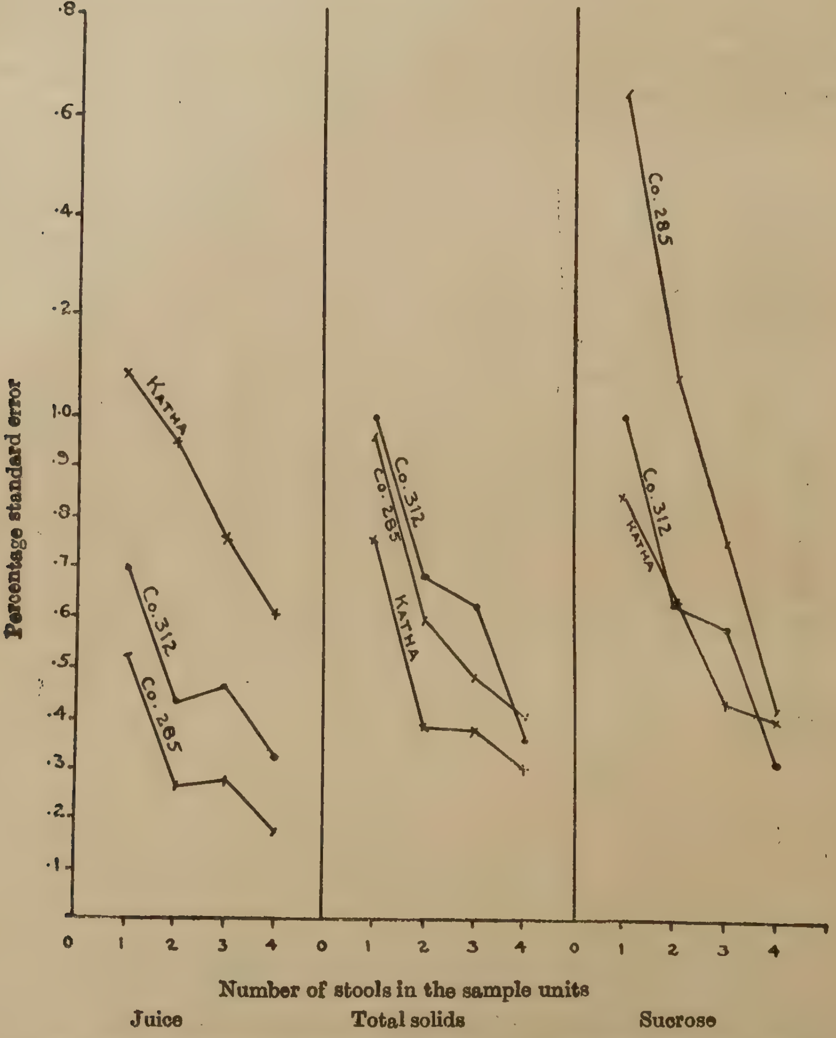


FIG. 2. Percentage standard errors for different varieties of sugarcane with regard to their different constituents—juice, total solids and sucrose.

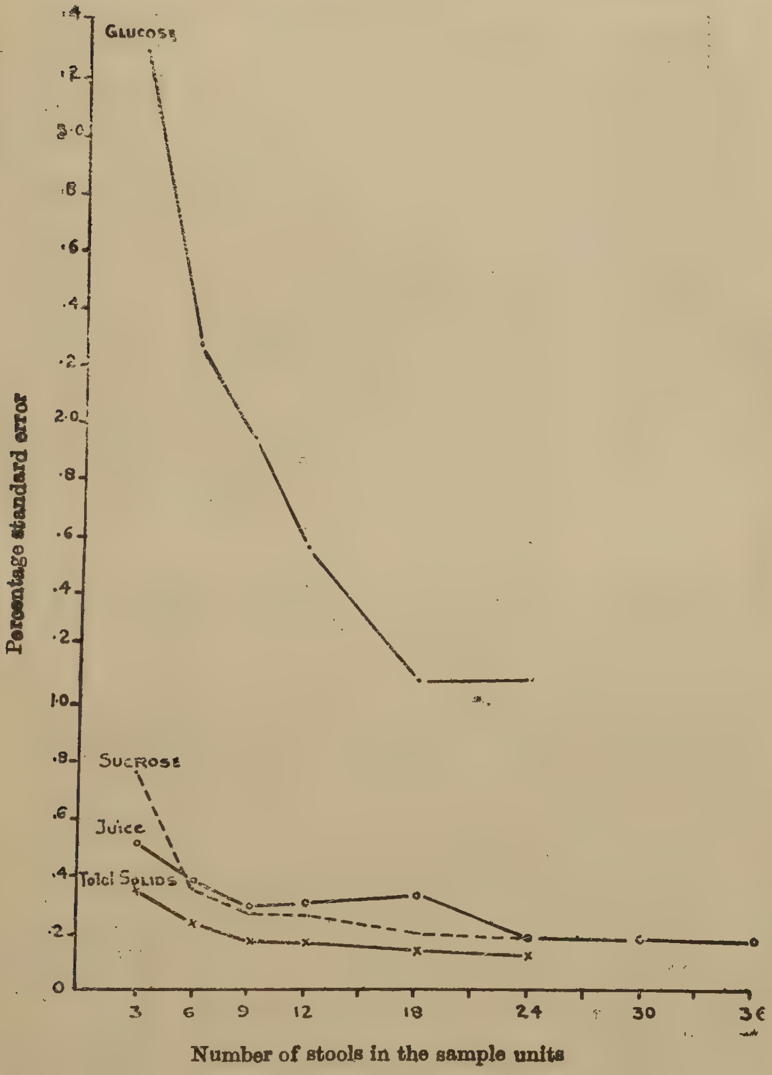


Fig. 3. Percentage standard errors for different constituents of Co 318

TABLE IV

Showing the mean values, standard deviations, standard errors and the coefficients of variation for different constituents of sugarcane from Montgomery and Lyallpur

Description	Juice per cent				Total solids (per cent)				Sucrose (per cent)				Glucose (per cent)			
	Mean	Standard deviation	Standard error (S. E.)	S. E. (per cent)	Mean	Standard deviation	Standard error (S. E.)	S. E. (per cent)	Mean	Standard deviation	Standard error (S. E.)	S. E. (per cent)	Mean	Standard deviation	Standard error (S. E.)	S. E. (per cent)
	Montgomery (16-sample units in the series)															
Katha																
1 stool samples	59·7	2·60	0·650	1·088	20·69	0·613	0·153	0·740	17·36	0·588	0·147	0·850				
2 ,, ,,	60·0	2·27	0·567	0·945	20·59	0·315	0·079	0·383	17·22	0·480	0·120	0·693				
3 ,, ,,	59·9	1·82	0·455	0·759	20·61	0·310	0·077	0·376	17·25	0·329	0·082	0·475				
4 ,, ,,	59·7	1·43	0·358	0·600	20·59	0·245	0·061	0·297	17·26	0·312	0·078	0·451				
Co 285																
1 stool samples	69·5	1·46	0·366	0·527	16·60	0·639	0·160	0·962	12·29	0·808	0·202	1·641				
2 ,, ,,	69·5	0·72	0·180	0·259	16·63	0·390	0·098	0·587	12·31	0·532	0·133	1·080				
3 ,, ,,	69·1	0·76	0·189	0·274	16·60	0·318	0·080	0·480	12·28	0·375	0·099	0·803				
4 ,, ,,	69·5	0·48	0·119	0·171	16·47	0·231	0·058	0·350	12·20	0·231	0·058	0·469				
Co 312																
1 stool samples	67·8	1·90	0·474	0·699	17·53	0·696	0·174	0·995	14·88	0·728	0·182	1·221				
2 ,, ,,	67·8	1·17	0·292	0·430	17·65	0·449	0·112	0·637	14·99	0·478	0·120	0·802				
3 ,, ,,	67·4	1·23	0·307	0·456	17·60	0·429	0·107	0·609	14·99	0·429	0·107	0·719				
4 ,, ,,	67·9	0·86	0·215	0·317	17·56	0·242	0·061	0·346	14·88	0·265	0·066	0·444				
	Lyallpur (48-sample units in the series)															
Co 318																
3 stool samples	62·5	2·22	0·320	0·512	19·95	0·482	0·070	0·349	15·30	0·585	0·084	0·552	0·368	0·084	0·012	3·315
6 ,, ,,	62·5	1·66	0·240	0·384	19·98	0·316	0·046	0·228	15·36	0·376	0·054	0·355	0·358	0·056	0·008	2·277
9 ,, ,,	62·7	1·26	0·182	0·290	19·97	0·232	0·034	0·168	15·35	0·279	0·040	0·263	0·359	0·049	0·007	1·958
12 ,, ,,	62·4	1·31	0·189	0·303	19·96	0·237	0·034	0·171	15·32	0·276	0·040	0·260	0·366	0·040	0·006	1·563
18 ,, ,,	62·7	1·42	0·205	0·327	19·96	0·173	0·025	0·125	15·33	0·200	0·029	0·189	0·362	0·028	0·004	1·097
24 ,, ,,	62·5	0·76	0·110	0·176	19·96	0·163	0·024	0·117	15·31	0·186	0·027	0·175	0·366	0·028	0·004	1·104
30 ,, ,,	62·5	0·75	0·108	0·173												
36 ,, ,,	62·4	0·69	0·100	0·160												

DISCUSSION OF RESULTS

Before entering into a detailed discussion of the various tables and figures presented, it may be mentioned that the idea of employing sampling methods for estimating the mean values of different constituents of a crop or yields of plots is not a new one. These have been used and are being used by a number of workers during the course of their daily routine work. The utility of such methods lies in the fact that the mean values of the sample whose properties have been found to represent those of the population, can be determined within reasonably small limits of error. By providing a sufficiently large number of repeats of samples of suitable sizes drawn at random from a normal population and examining these by statistical methods, it is possible to arrive at an estimate of the limits of error attaching to these values.

From the data for the Montgomery samples, it will be seen that the mean value for the juice percentage for any of the three varieties does not differ much in the case of sample series of different sizes. This means in other words that, as far as the figures for the juice percentage are concerned, a one-stool sample series gives as accurate an estimate of its mean value as any large sized sample series would do. If, however, we look at the figures for standard deviation and standard error we find these to decrease almost progressively with increase in the size of the sample. Thus in the case of Co 312 while the mean value for juice percentage does not differ by more than 0·5 in the case of different sized sample series the range of accuracy within which this mean value lies is considerably less in the case of sample series comprising four-stool sample units, *i.e.*, 67·9 ± 0·43 as compared with that of the one-unit sample series, where it is 67·8 ± 0·95, taking twice the standard error as the level of significance for the five per cent point. These points are brought out very vividly in Figs. 1 and 2. It will be seen that there are great variations in the percentage standard errors of different constituents in the case of all the three varieties when the size of the sample unit is one stool. While in the case of Co 285 and Co 312 the greatest variation occurs in the figures for sucrose, in the case of Katha on the other hand the figures for juice show the greatest variation. There is a clear indication that, with increase in the size of the sample unit above one stool, there is a rapid decrease in error till a four-stool sample unit is reached. This tendency for the percentage standard errors to come nearer to each other and coalesce or group together as the size of the sample unit increases, is to be noticed in the case of all the constituents, although in the case of the juice this grouping is not so pronounced. Another interesting observation which can be made from a study of the figures is that the percentage standard errors come nearer to each other with increase in the size of the sample unit not only for any one particular constituent in different varieties but also for different constituents of the same variety This may be interpreted to mean that in the smaller sized sample units the accuracy with which different constituents of any variety can be estimated differs widely, these differences, however, tending to decrease as the size of the sample unit is increased. This increase in accuracy definitely indicated the possibility of reducing the error still further by increasing the size of the sample unit. Consequently at Lyallpur, as has been mentioned already, the unit of sampling adopted was three stools. On examination of the Lyallpur data from which percentage standard error figures have been represented graphically in Fig. 3, we again find that for getting an accurate estimate of the mean values for various constituents, there is no advantage in increasing the size of the individual sample unit beyond three stools when the series consists of forty-eight sample units. In actual practice however, this will mean analysing forty-eight samples of three stools each, which evidently means a large amount of work. An attempt has therefore been made to study the possibility of obtaining a tolerable limit of accuracy with a reasonable lower number of sample units in the sample series. From a random selection out of the forty-eight original units given in Table III another series of samples consisting of ten, twenty, thirty and forty sample units of various sizes were constructed. The results are given in Table V and they supply interesting information. It will be observed that for all the three constituents for which the data have been worked out, *viz.*, juice, sucrose and total solids, the error for sample units of all the different sizes increases uniformly with decrease in the number of individuals in the sample series. Similar results are obtained by keeping the number of individuals in the series the same but increasing their size. It may, however, be remarked that in the case of juice the order of variations in the extent of error is not quite so regular as it is in the case of sucrose and total solids.

TABLE V

Showing the mean values, standard deviations and standard errors for different constituents of Co 318 in sample series composed of varying number of sample units of different sizes

Sample size	10-sample units				20-sample units				30-sample units				40-sample units				48-sample units			
	Mean	Standard deviation	Standard error	S. E. per cent	Mean	Standard deviation	Standard error	S. E. per cent	Mean	Standard deviation	Standard error	S. E. per cent	Mean	Standard deviation	Standard error	S. E. per cent	Mean	Standard deviation	Standard error	S. E. per cent
Percentage juice																				
3 stools .	62·7	3·64	1·500	1·830	62·9	2·74	0·612	0·973	62·7	2·33	0·425	0·678	62·5	2·18	0·344	0·550	62·5	2·22	0·320	0·512
6 ,, .	62·0	1·92	0·607	0·979	62·2	1·92	0·429	0·689	62·2	1·72	0·314	0·505	62·4	1·70	0·268	0·429	62·5	1·66	0·240	0·384
9 ,, .	62·8	1·30	0·400	0·637	62·8	1·21	0·271	0·432	62·8	1·28	0·233	0·371	62·6	1·20	0·190	0·304	62·7	1·26	0·182	0·290
12 ,, .	62·2	0·89	0·282	0·453	62·4	1·41	0·315	0·504	62·6	1·41	0·258	0·412	62·5	1·31	0·207	0·331	62·4	1·31	0·189	0·303
18 ,, .	63·0	1·55	0·489	0·776	62·5	1·98	0·442	0·707	62·5	1·68	0·306	0·490	62·6	1·51	0·237	0·379	62·7	1·42	0·205	0·327
24 ,, .	61·9	0·69	0·218	0·352	62·3	0·76	0·170	0·273	62·4	0·79	0·145	0·232	62·4	0·70	0·111	0·178	62·5	0·76	0·110	0·176
30 ,, .	62·4	1·04	0·329	0·527	62·5	0·84	0·189	0·301	62·5	0·60	0·110	0·176	62·5	0·60	0·094	0·150	62·5	0·75	0·108	0·173
36 ,, .	62·4	0·70	0·221	0·354	62·5	0·57	0·127	0·203	62·4	0·61	0·112	0·179	62·4	0·77	0·122	0·195	62·4	0·69	0·100	0·160
Percentage sucrose																				
3 ,, .	15·19	0·60	0·190	1·247	15·20	0·56	0·125	0·823	15·22	0·61	0·112	0·736	15·29	0·60	0·095	0·620	15·30	0·59	0·084	0·552
6 ,, .	15·33	0·45	0·143	0·987	15·34	0·41	0·092	0·602	15·33	0·38	0·069	0·452	15·34	0·39	0·062	0·404	15·36	0·38	0·054	0·355
9 ,, .	15·29	0·30	0·094	0·619	15·32	0·31	0·069	0·451	15·33	0·29	0·052	0·344	15·34	0·27	0·043	0·281	15·35	0·28	0·040	0·263
12 ,, .	15·21	0·27	0·085	0·560	15·29	0·28	0·063	0·416	15·34	0·28	0·050	0·330	15·57	0·25	0·040	0·261	15·32	0·28	0·040	0·260
18 ,, .	15·41	0·14	0·043	0·286	15·35	0·20	0·046	0·299	15·33	0·20	0·036	0·239	15·33	0·21	0·033	0·215	15·33	0·20	0·029	0·189
Percentage total solids																				
3 ,, .	19·91	0·50	0·158	0·796	19·84	0·46	0·110	0·553	19·73	0 50	0·091	0·463	19·73	0·46	0·073	0·368	19·95	0·48	0·070	0·349
6 ,, .	19·95	0·37	0·117	0·583	19·99	0·33	0·075	0·373	19·95	0·32	0·056	0·282	19·99	0·33	0·052	0·262	19·98	0·32	0·046	0·228
9 ,, .	19·97	0·23	0·072	0·362	19·97	0·22	0·052	0·260	19·93	0·23	0·041	0·207	19·94	0·22	0·034	0·172	19·97	0·23	0·034	0·168
12 ,, .	19·88	0·25	0·078	0·393	19·90	0·23	0·052	0·260	19·97	0·24	0·045	0·223	20·00	0·22	0·034	0·172	19·96	0·24	0·034	0·171
18 ,, .	20·02	0·19	0·061	0·306	19·93	0·19	0·041	0·208	19·92	0·17	0·032	0·159	19·88	0·16	0·025	0·126	19·96	0·17	0·025	0·125

Another interesting conclusion which emerges from a consideration of the data presented in this table is that a greater gain in precision is obtained by increasing the number of sample units in the sample series than by an increase in the size of the sample units, the total number of stools used being the same in either case. In order to make this point clear, Table VI given below has been derived from the previous table by calculating the proportionate standard error of ten, twenty and thirty individual sample units in the series when the percentage standard error for a sample series consisting of forty sample units has been fixed at 100. It will be seen for instance, that in the case of juice when the total number of stools dealt with is sixty, the error for three-stool sample units ($n = 10$) falls from 333 to 177 when the value of n is doubled and to 228 when the size of the sample unit is doubled. The same is the case for other combinations consisting of an equal number of stools. Similar relationship is also found to exist for sucrose and total solids.

TABLE VI

Showing relative percentage standard error of sample series composed of varying numbers of sample units of different sizes

Sample size	No. of sample units in the series			
	10	20	30	40
Juice				
3 stools	333	177	123	100
6 ,, ...	228	161	118	100
9 ,, ...	210	142	122	100
12 ,, ...	137	152	124	100
Total solids				
3 stools	216	150	126	100
6 ,, ...	223	142	108	100
9 ,, ...	210	151	120	100
12 ,, ...	228	151	130	100
Sucrose				
3 stools	201	133	119	100
6 ,, ...	244	149	112	100
9 ,, ...	220	160	122	100
12 ,, ...	215	159	126	100

The smallest sized sample, *i.e.*, of three stools which is subject to maximum variations has for a mean value of 15·19 for sucrose an error of 0·38 when only ten sample units in the series are provided. This limit has, however, been arrived at from an examination of only one set of figures. To see the limits of variation obtained by repeating the same method of sampling when the value of n is equal to ten in each of the four repeats provided, results presented in Table VII were worked out for sample units of all the eight different sizes. Although complete data have been worked out but for the sake of brevity only the average figures with their percentage standard error are given. These show that the lowest and highest percentage standard error figures stand very widely apart in the case of three-stool sample units but this difference tends to decrease as the size of the sample unit increases. Fig. 4 clearly brings out these differences. It will be seen that amongst the four repeats the greatest difference between the percentage standard error for any two sets is met with in the smallest sized sample. The diminution in the extent of the error, however, which is very great as we pass from three to nine stool sample units becomes appreciably smaller on passing from nine to eighteen stool sample units, and as far as juice is concerned beyond eighteen-stool sample unit size no appreciable gain in precision is obtained. Considering the columns (Fig. 4) representing the standard error for nine-stool sample units, it will be seen that the variation in the standard error of the four repeats are almost of the same order as those which are found to exist in the repeats of twelve-stool sample units. The mean value for different constituents are found to lie within the limits given in the last column of Table VII, which also gives the average figures for the standard deviation and standard error. In routine analysis, however, a sample of abnormally great size is as inconvenient to deal with as say ten replicates of small-sized samples. In the first case it is very difficult to maintain uniform conditions of milling throughout the entire period of crushing of a large-sized sample and this affects the figures for juice percentage considerably. In the other case the analyses of a large number of replicates of the same sample at the same time precludes the possibility of dealing with the large numbers of different samples which are usually received in the Laboratory for analysis on any one day. Therefore a single sample of such a size as would give us values for the different constituents of the cane lying within a desired range of accuracy will be more desirable. We have already seen that a considerable decrease in the percentage standard error takes place by correspondingly increasing the size of the sample. From the data given in Table IV, it is possible to determine the extent to which we can depend upon the results of the analysis of a single sample unit whose size has been increased to a reasonable workable limit. Considering the standard error obtained for sample units of various sizes we find that as we pass from a three-stool sample unit to one consisting of nine stools, in the case of juice and its constituents, there is a fall of about fifty per cent in the percentage standard error. In the case of juice a further increase in size up to eighteen stools results in no additional gain in precision. However, when the size of the sample units is increased to twenty-four stools, the error is again reduced to half and is now one-third of that for a three-stool sample unit. It continues at this level up to a thirty-six-stool sample unit. In the case of total solids, sucrose and glucose, there is comparatively a more regular diminution in error as the size of the sample unit is increased to twenty-four stools, although this gain in precision is not commensurate with the amount of increase in the size of the sample. The case of glucose which, as has been mentioned already, was estimated only at Lyallpur, deserves special mention. Of all the determinations for different constituent of cane, this is subject to the greatest error which, although it decreases with increase in the size of the sample unit, remains about six times that for juice or sucrose and nearly ten times that for total solids for all the different-sized sample units.

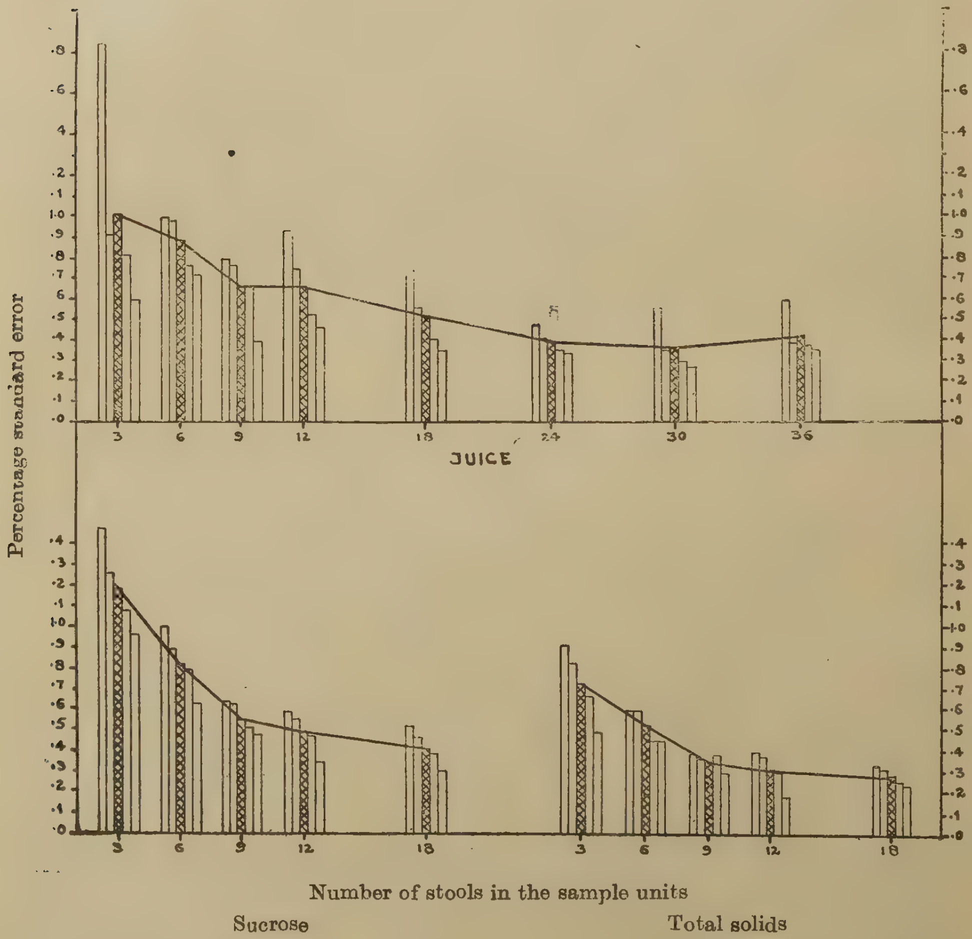


FIG. 4. Percentage standard errors in four replicates sample series each comprising ten sampling units of varying sizes together with the average S. E. per cent of the four replicates (shaded).

TABLE VII

Showing the average figures for juice, sucrose and total solids together with their statistical constants for four replicate sample series

Size of the sample unit	4 sample series each consisting of 10 sample units								Average of all the 4 repeats			
	1		2		3		4					
	Average	Percentage standard error	Average	Percentage standard error	Average	Percentage standard error	Average	Percentage standard error	Average	Standard deviation	Standard error	Percentage standard error
					Juice							
3 stools . .	62·7	1·830	63·1	0·796	62·2	0·582	61·9	0·896	62·5	2·03	0·642	1·026
6 ,, . .	62·0	0·979	62·5	0·990	62·2	0·721	63·1	0·751	62·5	1·71	0·537	0·860
9 ,, . .	62·8	0·637	62·5	0·768	62·8	0·764	62·0	0·390	62·5	1·27	0·400	0·640
12 ,, . .	62·2	0·453	62·6	0·921	63·0	0·730	62·3	0·505	62·5	1·29	0·413	0·652
18 ,, . .	63·0	0·776	62·1	0·404	62·5	0·539	62·9	0·334	62·6	1·02	0·322	0·513
24 ,, . .	61·9	0·352	62·7	0·320	62·6	0·462	62·6	0·391	62·5	0·75	0·238	0·381
30 ,, . .	62·4	0·527	62·5	0·328	62·6	0·259	62·3	0·284	62·5	0·69	0·218	0·350
36 ,, . .	62·4	0·354	62·6	0·324	62·3	0·366	62·1	0·578	62·4	0·80	0·253	0·405
					Total solids							
3 stools . .	19·91	0·796	19·94	0·653	19·93	0·898	20·01	0·482	19·95	0·297	0·141	0·707
6 ,, . .	19·95	0·583	20·04	0·435	19·60	0·438	20·04	0·578	19·91	0·215	0·102	0·509
9 ,, . .	19·97	0·362	20·00	0·361	19·96	0·369	20·01	0·288	19·99	0·143	0·069	0·345
12 ,, . .	19·88	0·393	20·02	0·299	20·07	0·392	20·03	0·159	20·00	0·131	0·062	0·311
18 ,, . .	20·02	0·306	19·91	0·250	19·99	0·229	19·96	0·326	19·99	0·117	0·056	0·278
					Sucrose							
3 stools . .	15·19	1·247	15·21	1·069	15·27	1·464	15·50	0·950	15·29	0·572	0·181	1·182
6 ,, . .	15·33	0·987	15·36	0·779	15·31	0·622	15·38	0·877	15·35	0·386	0·122	0·816
9 ,, . .	15·29	0·619	15·36	0·628	15·34	0·501	15·38	0·456	15·34	0·269	0·084	0·551
12 ,, . .	15·21	0·560	15·37	0·577	15·45	0·466	15·40	0·330	15·36	0·233	0·074	0·483
8 ,, . .	15·41	0·286	15·29	0·500	15·30	0·382	15·32	0·436	15·33	0·194	0·061	0·401

Although glucose possesses the greatest amount of error, but, since sucrose is the most important constituent of cane for judging its quality, the limits of accuracy of our analytical figures may well be considered sufficient, if they fall within the limits of error fixed for sucrose, the errors for juice and total solids being well within these limits.

According to Leather [1913], " with the experience gained at Pusa over three seasons, and at Partabgarh as well as with the West Indian canes, it appeared probable that a much higher degree of accuracy than ± 0·5 per cent of sucrose would be difficult to secure ". A reference to Table IV will, however, show that the range of variation can be made much less than ± 0·5 per cent. The lowest limit that we have worked out for a sample series consisting of forty-eight sample units of twenty-four stools each, is 0·027 which in terms of Leather's method of representation (at five per cent point) means a variation of ± 0·054, and, from the data supplied in this paper, this could be worked out for still larger-sized sample units, but the gain in precision would not be commensurate with the trouble and inconvenience involved in handling so many inordinately larger-sized sample units in the series. It would therefore seem that for a sucrose variation of ± 0·5 we can reduce the value of *n* considerably below forty-eight. For a nine-stool sample unit the error for sucrose as seen already is 0·279 or ± 0·558 according to Leather's method of expression. This value is still less in the case of total solids. This means that as far as the quality of the juice—as judged from its composition—is concerned, we can get a reasonably correct estimate by analysing one composite sample consisting of nine to ten stools, and this will give us almost as accurate an estimate of the percentage amounts of different constituents of the juice as a sample consisting of 160 or 200 individual canes picked up according to Leather's method. It will, however, be advisable to employ a much larger sample if the results are to be expressed as percentages on the weight of cane which means the same thing as expressing the yields of different constituents per acre calculated on the basis of the total amount of juice extracted. This, as we have already explained, is due to the figures for juice percentage being dependent not only on the quality of the cane but also on other extraneous factors, *i.e.*, the milling conditions. Figures in Table VIII, which has been prepared from the Montgomery data given in Table II, confirm this observation. The percentage standard error of the sample series comprising sixteen one-stool sample units for Co 285 and Katha, which, of the three varieties analysed at this station, have shown the smallest and the greatest errors for juice, *viz.* 69·5 ± 0·366 and 59·7 ± 0·650 respectively, have been calculated after converting the sucrose figures as percentages on cane.

TABLE VIII

Showing the mean values and percentage standard error of sucrose expressed as percentage on juice as well as on cane

Variety	Per cent sucrose on juice		Per cent sucrose on cane	
	Mean value for sucrose	Percentage standard error	Mean value for sucrose	Percentage standard error
Co 285 . . .	12·3	1·641	8·53	1·690
Katha . . .	17·3	0·849	10·60	1·34

It will be seen from this table that the error for sucrose in both the varieties is liable to increase when the data are expressed as percentages on cane instead of on juice, since errors due to variations in the milling conditions—though they may be only of a small magnitude when proper precautions are observed during crushing—will also be included. Therefore in the case of experiments designed to compare the quality of cane as influenced by different treatments, rather than its yields we would recommend that the results of analysis be reported as percentages on juice. We have seen that for the purpose of comparing two or more varieties of sugarcane or for studying their response to different treatments, a sample consisting of nine or ten stools, will give as accurate an estimate of the amount of different constituents of the cane as one could wish in any scheme of routine analysis. In experiments requiring greater accuracy or where the data obtained by analysis is to be expressed on a quantitative basis, *i.e.*, in terms of yields per acre, the above size of the sample, however, is not expected to give results of desired accuracy. A reference to Table IV will show that the standard deviation of juice for a sample unit consisting of nine stools is 1·26 and this, like percentage standard error which has already been dealt with, remains almost constant for all sample units up to the limit of eighteen stools. For a twenty-four-stool sample unit there is again a fall in the standard deviation, which now continues at almost the same level up to a thirty-six-stool sample unit. For five per cent level of significance, the limits of variation for juice, total solids, sucrose and glucose, in the case of a twenty-four-stool sample unit, are 1·5, 0·33, 0·37 and 0·06 respectively. These limits of error can be reduced further by increasing the size of the sample unit but the gain in precision is not proportionate. Hence we consider the above limits good enough for all accurate and quantitative work.

The limits of accuracy obtainable with nine and twenty-four-stool samples as given above have been worked out in the case of Co 318. This variety was analysed rather late in the season, *i.e.*, about the end of March, which under local conditions is regarded as the usual limit up to which the cane crop may be allowed to remain standing in the field without suffering any deterioration in its quality. The conclusions based on the results of analyses of the Lyallpur samples may, therefore, be assumed to cover a sufficiently wide range of variations which can be expected to occur in the different constituents of cane during any crushing season. As far as the composition of their juice is concerned, Lander and Ramji Narain [1935] found that under abnormal conditions different cane varieties react differently to the onset of severe frost or attacks of certain diseases or pests such as red-rot, *Pyrilla*, etc. These we propose to examine later on. But under normal conditions, in all those special cases, where, with regard to any particular variety or treatment a still more accurate estimate of errors for the different constituents of the juice is required, it can be obtained by providing a number of repeats. It would, however, not be always necessary to take a sample series with as many sample units (forty-eight) as had been tentatively done by us for working out the data presented in this paper. All that is required is first to decide upon the limits of accuracy the experimenter desires to attain for the purpose of his investigations and then to select from the results given in Table V the most suitable size of the sample unit as well as the required number of replications that would give the values of various constituents within the desired range and yet be not inconveniently large. We are of opinion that for this purpose ten samples of ten stools each which in practice can be conveniently dealt with will meet the requirements fairly well. The limits of accuracy obtained under these conditions are given in the last column of Table VII, and, as will be seen, these as far as the quality of the juice is concerned are about half the figures of errors for different constituents obtained with a single sample of twenty-four stools.

The conclusions arrived at have been reached from a consideration of the data pertaining to Co 318 grown at Lyallpur during the season 1935-36. How far these will apply to this and other varieties grown at other stations during different seasons will be examined later on.

SUMMARY

1. The investigation was undertaken with the object of (*i*) selecting the limits of accuracy within which the mean value of different constituents of sugarcane from any plot should lie so as to yield reliable information about the condition of the crop as influenced by different treatments and (*ii*) fixing the size and manner of taking the sample for analysis which would be subject to an error not greater than the limits of tolerance so fixed.

2. The factors influencing the results of analysis have been briefly discussed and it has been shown that while the most important of these is sampling, considerable errors are liable to result from variations in the conditions of extraction.

3. Data from the chemical analysis of three varieties of cane from Montgomery and one from Lyallpur have been used to construct different sample serie made up of sample units of various sizes. The values for different constituents of sugarcane thus obtained for samples of varying sizes, have been examined statistically at considerable length and from the constants so obtained the limits of accuracy for the mean value of different constituents of cane which may be tolerated have been determined.

4. It has been found that variations in the different constituents of the same variety as well as similar constituents of different varieties tend to decrease with the increase in the size of the sample.

5. Data have been presented which can be used to determine the size of the sample which will give analytical figures lying between limits of accuracy desired by the investigator. It has been shown that for ordinary analysis of sugarcane juice, a sample consisting of ten stools picked at random from the entire field will give as accurate results as may be required in routine analysis. In special cases where still greater accuracy is desired ten replicate samples each consisting of ten stools will meet requirements fairly well. If, however, the results are to be expressed as percentage on cane and also when a reliable estimate of juice percentage is required, a sample consisting of twenty-five stools will be necessary.

6. The figures for sucrose and total solids have been found to be accurate within a range of about ± 0·5 and for glucose within ± 0·10 when a nine-stool sample is taken, and those of juice percentage within ± 1·5 with a twenty-four-stool sample. The limits of accuracy for the mean of ten samples of nine stools each will be ± 1·3, ± 0·14, and ± 0·17 for juice, total solids and sucrose respectively.

We have great pleasure in acknowledging our thanks to Bh. Balmokand, M.Sc., Assistant Professor of Physics and Mathematics for his helpful criticism and valuable suggestions in the preparation of this paper.

REFERENCES

Clarke, G. and Hadi, S. M. (1908). *Agric. Res. Inst., Pusa, Bull.* No. **13**.

Croucher, H. H. (1933). *Annual Rept. Dept. Agric., Jamaica.*

Davis, R. L. (1934). *Agric. Notes, Puerto Rico Agric. Expt. Station, Mayaguez,* No. **67**.

Dymond, G. C. (1935). *Proc. So. African Sugar Tech. Assoc.* **9**.

Elderton, W. P. (1927). 'Frequency Curves and Correlation', 2nd edition.

Fisher, R. A. (1932). 'Statistical Methods for Research Workers', Oliver and Boyd.

Kobus, (1906). *Int. Sugar Jour.* Nos. **90**, **91**.

Lander, P. E. and Ramji Narain (1935). *Ind. Jour. Agric. Sci.* **5**, Part II.

Leather, J. W. (1913). *Mem. Dept. Agric. Ind.* (*Chem. Ser.*) **3**.

Moberly, G. S. (1935). *Proc. 9th Congress of So. African Sugar Tech. Assoc.*

RELATIVE SUSCEPTIBILITY OF SOME WILD AND CULTIVATED POTATO VARIETIES TO AN EPIDEMIC OF LATE-BLIGHT AT SIMLA IN 1936

BY

B. B. MUNDKUR,

B. P. PAL

AND

PUSHKAR NATH

Imperial Agricultural Research Institute, New Delhi

(Received for publication on 16th February 1937)

(With Plate LII)

INTRODUCTION

PROBABLY no single disease of the potato crop is so destructive and causes such a great reduction in yield of tubers as the late-blight due to *Phytophthora infestans* (Montagne) de Bary. This is especially so in regions with a temperate climate, particularly those of Europe and North America. In years when the disease is in its worst form, there may be no tubers to harvest.

In the plains of India where potatoes are grown as a winter crop (October-March) the disease, according to Dastur [1915], is relatively rare. But in Assam and the foot-hills of the Himalayas, at elevations ranging from four to ten thousand feet above the sea level, where a large area is under potatoes, late-blight occurs very frequently. As potatoes form the principal and sometimes the only crop that can be raised on these mountain slopes, a visitation by this disease is dreaded by the potato-growers. It considerably affects the market price in the plains, for the crop raised in the plains is not adequate to meet the demand. The seed-potato supply of the plains, furthermore, needs to be augmented and periodically replenished by seed from the hills. Not only is this supply affected but in years when moisture and temperature are favourable, the tubers from the hills may harbour the dormant mycelium of the late-blight fungus and may cause epidemics in the plains. Late-blight epiphytotics thus constitute an important factor in potato cultivation, both directly and indirectly, in this country, and in the project* for the improvement of Northern India potatoes, a study of this disease, leading towards its control, is one of the major problems.

---

* This project is financed by the Imperial Council of Agricultural Research to whom thanks are hereby rendered for the facilities provided.

In 1936 the disease appeared in a severe form at Simla and provided an opportunity for making detailed observations of the symptoms, studying the fungus in a preliminary way to confirm its identification and determining the relative susceptibility of several varieties of wild and cultivated potatoes that were growing at the Potato Breeding Sub-Station at that place. This note is preliminary to others which it is hoped to publish as investigations progress.

NATURE OF ATTACK

Symptoms.—Rains started a month earlier than is usual for Simla in 1936 and July was especially wet and cloudy in the latter part of the month. Mean day and night temperatures were lower than usual and these factors seem to have favoured the appearance of late-blight which was first observed on the plants growing at the Sub-Station on the 23rd of July. Small yellowish spots which later turned brown, first manifested themselves on the under-side of the leaflets. They were surrounded by a whitish glistening ring so long as the weather was wet but with the appearance of drier conditions, this ring either became faint or disappeared entirely. The spots extended quickly over the entire surface of the leaves, many of which hung loosely or dropped down. The rot was first a wet rot but later became a dry rot and the plants presented a blackish appearance.

It was noted that the lower leaves first showed the symptoms which slowly spread upwards ; the entire plant was ultimately involved. The petioles were invariably rotted but the stem was not always attacked. As will be evident later, it was only in the most susceptible varieties that the stems also completely rotted. As the disease progressed upwards, the pedicels decayed and the flowers dropped down ; where berries had formed, they were also attacked. A large number of tubers which had commenced to form, rotted also, but the rot was invariably a dry rot characteristic of *P. infestans* and not the wet rot caused by bacteria and other causes.

Within a week's time the disease took a serious turn and the crop which was green and healthy became a mass of decayed foliage hanging about the stems. The whole of August continued to be cloudy and the disease spread rapidly, inflicting much damage.

The causal organism.—Microscopic examination of the newly formed leaf-spots indicated that within the mesophyll of the leaf tissues was a richly branched, inter-cellular, hyaline mycelium from 3 to $4 \cdot 5\mu$ in thickness. The mycelium was filled with actively streaming protoplasm and the thickness of the mycelium and the absence of septa left no doubt that it was a phycomycete. A scraping from the whitish ring was full of sporangia. The mycelium was traced in the tissues of the petiole and the stem but the fruiting bodies were formed only on the surface.

Isolation of the fungus.—No facilities being then available at the Sub-Station for isolating the fungus, newly attacked leaves, petioles and stems were brought to Delhi in September. They were cleaned by washing in several changes of sterile water, placed in sterile test-tubes, corked and sealed with paraffin. The tubes were then placed in a packing of ice within a wide-mouthed thermos flask and stored. *Phytophthora infestans* can be isolated only when the temperature of the atmosphere is around 22°C. or below. No attempt was made to isolate the fungus at that time as the atmospheric temperature at Delhi was much higher.

In the third week of November the temperature of the atmosphere became rather favourable and the isolation of the fungus was immediately proceeded with. Surface-sterilised pieces of tissue placed aseptically on Clinton's oat-juice agar showed mycelial strands on the sixth day. Cottony aerial growth in appreciable quantity was visible on the twelfth day. A microscopic examination at this time indicated that fruiting bodies, sporangiophores and sporangia, had already commenced to form. Out of seventeen test-tubes growth appeared in eleven, two others were contaminated and there was no growth in the rest. The fungus was transferred to fresh test-tubes containing oat-juice agar to which mashed prunes had been added at the time of making, at the rate of three prunes per litre of medium.

Morphology of the fungus.—The mycelium of the fungus is thick, unseptate, and well branched. The sporangia are lemon-shaped, compact, with a colourless membrane and a rather thickened apex. The cell contents are hyaline. The sporangiophores are sparingly branched and show a series of swollen joints which mark the position where sporangia that have fallen off were borne. This is a characteristic of *P. infestans* which is of diagnostic value. Sporangia germinate directly by means of germ-tubes or indirectly, by dividing up the protoplasm within the sporangium into zoospores. The former type of germination is more common at temperatures above 20°C., the latter at lower temperatures. Both the types of germination have been noted at Delhi. Zoospores emerged through an aperture at the apex of the sporangium and were motile for some time. Six to sixteen zoospores emerged from each sporangium. Germination of the zoospores has also been observed and this is by means of germ-tubes. No resting spores or zoospores have been observed in the material brought from Simla nor have haustoria been seen but the material was too scanty for a more detailed study.

A culture of *P. infestans* deposited by Giddings in the Central Bureau of fungal Cultures, Baarn, Holland, became available* early in December. This culture seems to be a descendant of the cultures isolated by Jones, Giddings and Lutman [1912] in Vermont. On comparing the Simla isolate with this culture, it was noted that our culture grew less vigorously on oat-prune agar and formed less aerial mycelium, but in all other respects it agreed with Giddings' isolate. Sporangial measurements of the two isolates growing on the same medium for twelve days were made and the frequency distributions compared. Neither in respect of length nor breadth (one hundred sporangia each) did the Simla isolate differ from Giddings' culture. The measurements are given below :—

---

* Grateful acknowledgment is made to Mr. S. F. Ashby, Director, Imperial Mycological Institute, Kew, for his kindness in furnishing this culture.

TABLE I

Sporangial measurements of the Simla and Giddings' P. infestans

	Giddings' isolate		Simla isolate	
	Range	Mean	Range	Mean
Length in μ	26—31	28·3±1·12	26—31	28·7±1·36
Breadth in μ	14—19	17·1±0·93	15—19	17·6±0·81

The Simla *Phytophthora* resembled therefore an authentic culture of *P. infestans* in all major characteristics including sporangial size, leaving no doubt regarding its identity.

SUSCEPTIBILITY OF *Solanum* SPP. AND POTATO VARIETIES

The recent work of Vowinckel [1926], Reddick [1928], Müller [1931] and Schick [1932] has made it clear that within the tuber-forming species of the genus *Solanum*, especially those occurring in a wild state in the mountain regions of Colombia, Peru and Mexico, there are varieties which show considerable variation in their susceptibility to *P. infestans*. Expeditions sent to these regions by Russia, Germany and the United States of America have resulted in securing material of blight resistant tuber-forming species. The work of Müller [1931] and Salaman [1932] has shown that even among the varieties of the common cultivated potato there are some which possess a certain amount of resistance to this disease. Investigations with these resistant varieties by Schick and Lehmann [1936] have further indicated that the pathogen itself is a composite species segregating into parasitic races which are, however, morphologically and physiologically indistinguishable.

Through the courtesy of the Russian, German, American and British Potato Breeders, and of Dr. P. S. Hudson of the Imperial Bureau of Plant Genetics, Cambridge, a large number of tuber-forming species as also varieties of the cultivated potato was obtained. The epidemic of 1936 which, as already stated

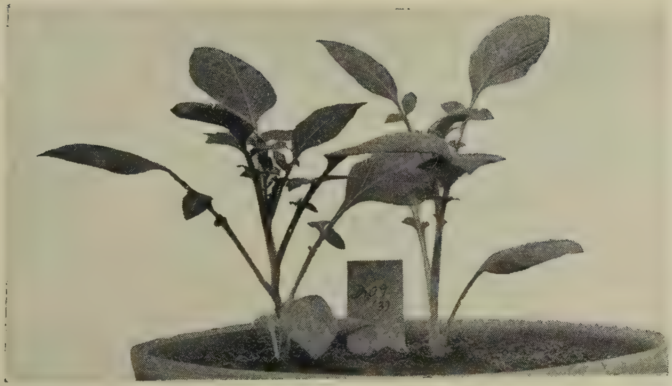


FIG. 1

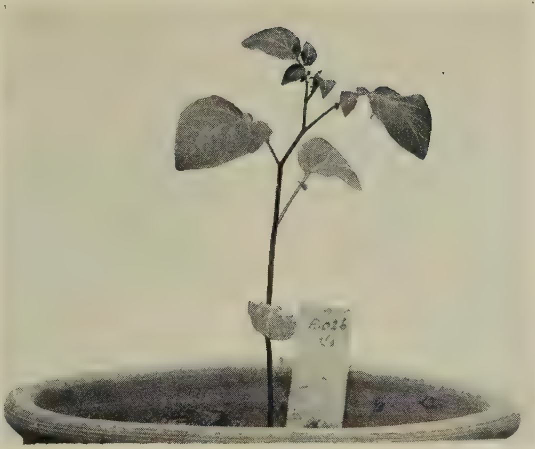


FIG. 2

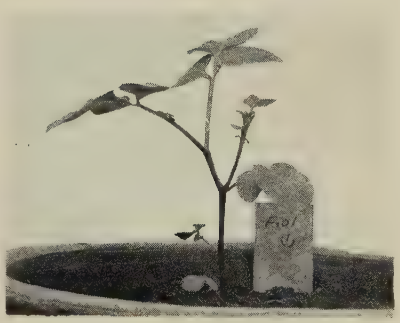


FIG. 3

FIGS. 1, 2 and 3. Seedlings of *S. demissum*, *S. neoantipoviczii* and *S. neoantipoviczii* var. *Reddickii*, respectively.

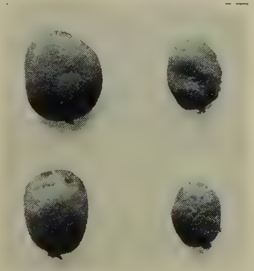


FIG. 4

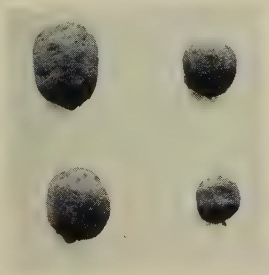


FIG. 5

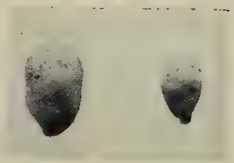


FIG. 6

FIGS. 4, 5 and 6. Tubers of ***S. demissum***, *S. neoantipoviczii* and *S. neoantipoviczii* var. *Reddickii*, respectively.

(Actual size)

was extremely virulent, provided an opportunity for determining the relative susceptibility of many of these varieties growing at Simla.

Examination of the different varieties with regard to their reaction to attack by the pathogen indicated that the resistance of leaves, stems, and tubers varies with the varieties. The foliage of most of the species was, however, always susceptible. Stems of some of the varieties were not always so but whenever the stems showed resistance, tubers were invariably free from attack. Some varieties possessed tubers that resisted the disease even though the leaves and stems were susceptible. The reaction of the various *Solanum* species to late-blight is recorded *seriatim* below :—

S. tuberosum L. (Cultivated European and Indian varieties).—A majority of the varieties were found to be highly susceptible ; some however did indicate a certain degree of resistance. These are :—

Fairly resistant :	President
Slightly resistant:	Pusa White, Gharwal, Khabrar, Conoor White, and Darjeeling Red.

S. tuberosum L. (South American varieties).—Out of one hundred and twenty-four varieties grown, none showed any immunity from the disease. About six varieties showed a high and promising degree of resistance but the rest were all very susceptible.

S. andigenum Juz. et Buk.—The majority of the varieties of this species were highly stem-resistant. The foliage in earlier stages also exhibited a high degree of resistance but later the foliage was attacked and there was a moderate degree of infection. The tubers appeared to possess a high degree of resistance as very few of them rotted.

S. demissum Lindl., *S. neoantipoviczii* Buk., *S. neoantipoviczii* var. *Reddickii* (Plate LII), *S. Antipoviczii* Buk.—These were immune from the disease. None of the plants were infected either in the fields or pots. Potted plants of these varieties with the exception of the last, were placed amidst heavily infected plants in a field for ten days. None of the plants caught infection.

S. Fendleri Gray, *S. Maglia* Schlecht., *S. otites*, *S. leptostigma* Juz.—These were very susceptible to the disease and the plants showed infection on the leaves, stems and tubers. All plants had completely died.

S. chacoense Bitt., *S. Caldsii glabrescens* Dunal and *S. Commersonii* Dunal.—Only the leaves showed high susceptibility to the disease but stems and tubers manifested a considerable degree of resistance. On being sprayed with Bordeaux, the stems put forth new growth which was luxuriant, and which produced a fresh crop of flowers and berries. Yield was good and tuber infection was absent.

The observations recorded above indicate that both in the common cultivated varieties of potatoes and among the wild species of *Solanum* there are varieties which show varying degrees of resistance to the race of *P. infestans* occurring at Simla. They also open up possibilities of developing new varieties by hybridising the present commercially desirable varieties of potatoes with those which show a high degree of resistance to late-blight.

SUMMARY

1. Late-blight caused by *Phytophthora infestans* appeared in a virulent form at Simla in 1936. July and August were especially wet and cloudy and this seems to have favoured the appearance and rapid spread of the disease.

2. The fungus was isolated at Delhi and a comparative morphological study of this isolate with an authentic culture of *P. infestans* showed that the Simla isolate agreed in all respects with the authentic isolate.

3. A large number of common cultivated potatoes secured from Europe and South America and also places in India were growing at the Sub-Station and were automatically exposed therefore to this severe blight infection. Some varieties showed a certain degree of resistance to the disease.

4. Several species of tuber-forming *Solanum* secured from abroad were also growing in the open at Simla. Many of these were immune from the disease while a few showed a very high degree of resistance.

5. The 1936 epidemic served therefore as a means to find out, under very severe infection conditions whether there were any varieties among the common cultivated potato which resisted the physiologic race of *Phytophthora infestans* occurring at Simla and to determine the resistance of other tuber-forming species of *Solanum*.

6. Preliminary observations indicate that there are resistant as well as highly resistant varieties which can be utilised in evolving types with the required economic qualities as well as resistance to late-blight.

REFERENCES

Dastur, J. F. (1915). *Mem. Dept. Agric. Ind.* (*Bot. Ser.*) **7**, 1-14.

Jones, L. R., Giddings, N. J. and Lutman, B. F. (1912). *Vermont Agric. Expt. Stn. Bull* **168**.

Müller, K. O. (1931). *Biol. Reichsanst. Land-u. Forstw. Arb.* **18**, 465-505.

Reddick, D. (1928). *Phytopath.* **18**, 483-502.

Salaman, R. N. (1932). *Deuxieme Congres Internationale de Pathologie Comparee*, pp. 436-437.

Schick, R. (1932). *Züchter.* **4**, 233-237.

Schick, R. and Lehmann, H. (1936). *Züchter.* **8**, 34-46.

Vowinckel, O. (1926). *Biol. Reichsanst. Land-u. Forstw. Arb.* **14**, 588-641.

THE GRAPE-VINE THRIPS (*RHIPIPHOROTHRIPS CRUENTATUS* HOOD) [THRIPIDAE: TEREBRANTIA: THYSANOPTERA]

A.M.L.

BY
KHAN A. RAHMAN, B.Sc. (Edin.), Ph.D. (Cantab.)
AND
NAND KISHORE BHARDWAJ, M.Sc. (Pb.)
Entomological Laboratory, Lyallpur

(Received for publication on 9th April 1937)
(With Plates LIII—LV)

CONTENTS

		Page
I.	Introduction	633
II.	Geographical distribution and food-plants	634
III.	Description of instars	634
IV.	Life-history	636
V.	Seasonal history	639
VI.	Parthenogenesis	639
VII.	Hibernation	639
VIII.	Progression on water	641
IX.	Submergence of adults	642
X.	Dispersal	642
XI.	Damage	643
XII.	Susceptibility of different varieties of grape-vine	645
XIII.	Resistance of adults to cold	645
XIV.	Parasite	646
XV.	Preventive measures	648
XVI.	Control measures	649
XVII.	Acknowledgments	650
XVIII.	Summary	651
	References	651

I. Introduction

Rhipiphorothrips cruentatus Hood,* a destructive pest of grape-vine, was first described by Hood [1919] as a new species from the specimens collected by the Entomologist to Government, Punjab, from grape-vine leaves at Lyallpur, and by Ramakrishna Ayyar from grape-vine leaves at Coimbatore and Bezwada, and *Eugenia jambolana* leaves at Bangalore [Karny, 1927].

---

* Kindly identified by Mr. Laing of the British Museum (Nat. Hist.), London.

II. GEOGRAPHICAL DISTRIBUTION AND FOOD-PLANTS

R. cruentatus is widely distributed throughout India, and feeds on a number of plants. The following table gives its food-plants together with localities (in India) from where it has been so far recorded :—

Locality	Food-plant	Reference
Lyallpur	Grape-vine, rose and *Terminalia arjuna* leaves.	Afzal Husain [1923].
South Arcot	*Odina wodier*	Karny [1927].
Madura	*Eugenia jambolana*	Karny [1927].
Coimbatore	Rose and grape-vine leaves, *Calotropis* flowers.	Karny [1927].
Bangalore	*E. jambolana* leaves	Ramakrishna Ayyar [1929].
Bezwada	Grape-vine leaves	Ramakrishna Ayyar [1929].
Coimbatore		
Madura		
Ootacamund	Grape-vine leaves	Ramakrishna Ayyar [1932].
Botanic gardens		
Not stated	Garden rose, *Terminalia catappa, Anacardium occidentale* and *Caryea arborea.*	Ramakrishna Ayyar [1932].
Peradeniya (Ceylon) (Rutherford).	Not stated	Ramakrishna Ayyar [1932].

III. DESCRIPTION OF INSTARS OF *Rhipiphorothrips cruentatus* HOOD

Egg (*Plate LIII, fig. 1*).—Length 0·26 mm., greatest diameter 0·12 mm. Beanshaped with slightly thicker basal end. Chorion ornamented with hexagonal markings. Greenish when freshly laid, but changes to dirty white later on ; the two red compound eyes of the nymph becoming visible a short time before hatching.

First instar nymph (*Plate LIII, fig. 2*).—Translucent when freshly hatched, but develops a pinkish tinge after twenty-four hours. Head pinkish brown ; compound eyes red, antenna (Plate LIII, fig. 2-*a*) 0·26 mm. long. Last two segments forming a style which bears a spine distally (Plate LIII, fig. 2, *st*) . Prothorax reddish brown, sides nearly parallel. Pterothorax light pink, antero-lateral ends reddish. Legs normal, tarsus of each leg terminating in a swollen air vesicle. Abdomen 10-segmented ; 1st to 7th abdominal segments light pink with a longitudinal reddish band along sides ; 8th red ; 9th and 10th light brownish, last segment with sixteen long black and stout apical spines arranged in a ring (Plate LIII, fig. 2, *s*).

RHIPIPHOROTHRIPS CRUENTATUS HOOD

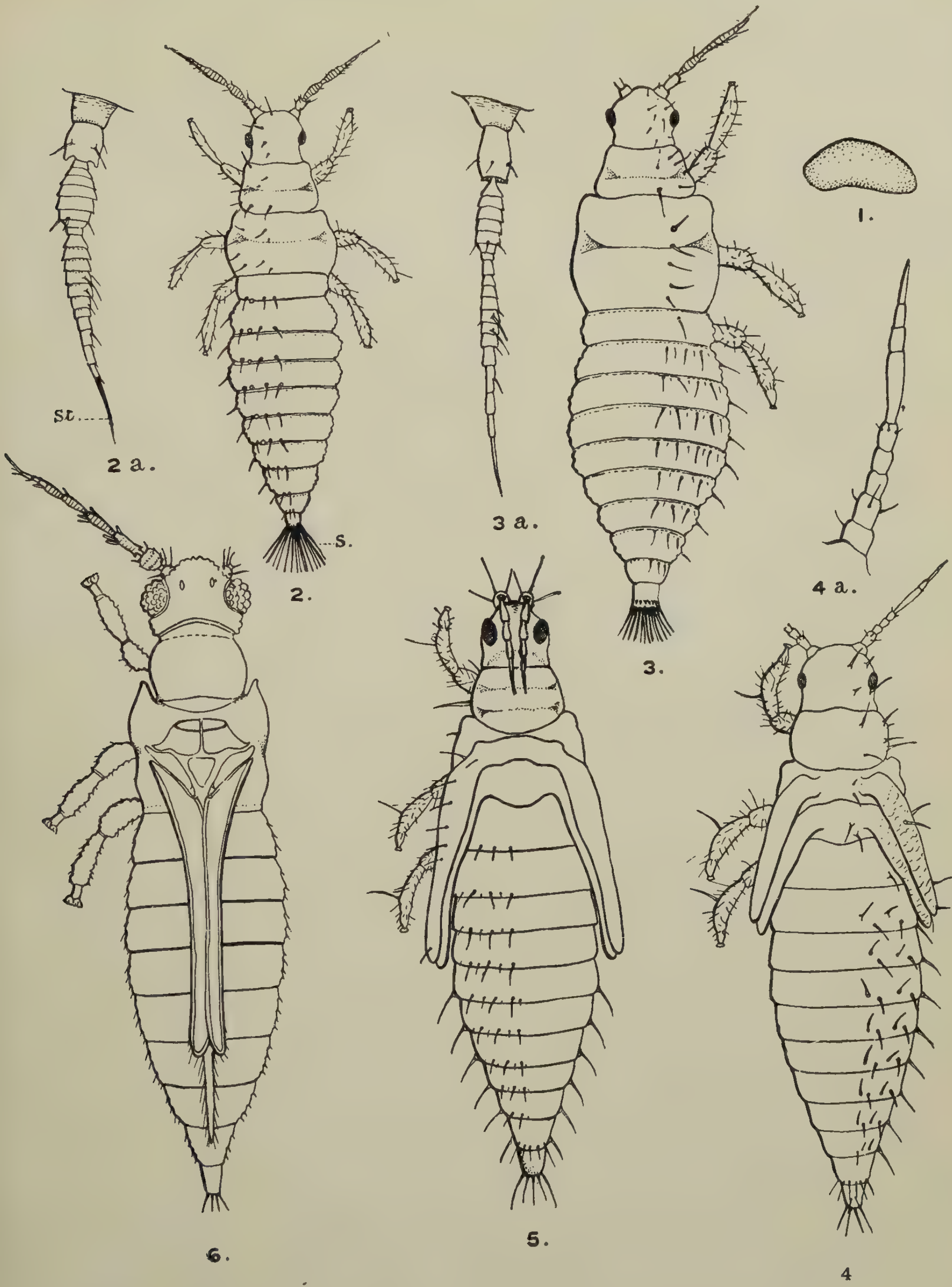


Fig. 1. Egg.
Fig. 2. First instar nymph. (Note caudal spines *s*).
Fig. 2a. Antenna of first instar nymph. (Note style *st.*)
Fig. 3. Second Instar nymph.
Fig. 3a. Antenna of second instar nymph.
Fig. 4. Prepupa.
Fig. 4a. Antenna of prepupa.
Fig. 5. Pupa.
Fig. 6. Female.

Second instar nymph (*Plate LIII, fig. 3*).—Yellowish white, with a reddish stripe on the prothorax, and with pinkish antero-lateral corners of pterothorax, and brick-red sides of each abdominal segment. Antenna (Plate LIII, fig. 3-*a*) 0·4 mm. long, otherwise similar to the antenna of the 1st instar nymph. Legs clothed with setae, longer than the legs of the subsequent instars. Abdomen longer and stouter as compared to the abdomen of the 1st instar nymph.

Prepupa (*Plate LIII, fig. 4*).—General colour and markings on the body similar to those of the 1st instar nymph. Head broader than long, sparingly clothed with setae. Compound eyes red. Antenna (Plate LIII, fig. 4-*a*) 0·22 mm. long, shorter than that of 2nd instar nymph. Prothorax with a number of small setae and eight strong spines. Pterothorax fairly well clothed with setae of varying sizes. Wing-pads whitish yellow ; anterior pair extending nearly to the 2nd abdominal segment, posterior pair extending a little further back. Front margin of anterior wing-pads with a few setae. Abdomen with caudal setae very much reduced in size and number.

Pupa (*Plate LIII, fig. 5*).—Light brown with 1st to 6th abdominal segments pink laterally and 7th to 10th segments pink all over. Head fairly thickly clothed with setae. Compound eyes red, larger and more prominent than those in the preceding instars. Antenna 0·34 mm. long, bent caudad at the 2nd segment, lying back over the head and prothorax. Prothorax broader than long, ornamented with crescentic markings. Pterothorax shield-shaped, divided into meso and metathorax by a prominent groove. Crescentic markings present. Wing-pads extending to the 5th abdominal segment. Legs armed with spines. Abdomen widest at the 3rd segment, caudal setae much reduced in size and number.

Adult (*Plate LIII, fig. 6*).—Hood [1919] describes the adult as follows :—

" *Female* (*Macropterous*).—Length about 1·4 mm., dorsal surface deeply reticulate. Colour of fully matured individuals dark blackish brown, with legs and antennae yellow and the head, dorsum of pterothorax laterad and caudad to scutellum, median third of abdomen, and last three abdominal segments distinctly paler ; 7th and 8th segments of antennae and apex of 6th segment more or less darkened with gray ; fore-wings yellowish, with costa and longitudinal veins slightly darker, and scale, posterior margin, and extreme base dark brown ; hind wings yellowish, with dark brown median vein. Teneral individuals paler than as above described and with the sides of head, thorax, and abdomen suffused with blood-red hypodermal pigmentation.

Head deeply rugoso-reticulate, about 1·21 times as wide as long and slightly longer than prothorax, very slightly broadest across eyes ; vertex strongly elevated and produced in front of eyes over insertion of antennae, notched anteriorly ; cheeks nearly parallel in basal half, swollen behind eyes and abruptly constricted at extreme base where inserted in prothorax, the anterior of this neck-like constriction marked with a prominent, raised, arcuate, dark carina. Eyes about equal in length to their distance from posterior margin of head and half as wide as their interval, strongly protruding in front, nonsetose. Ocelli nearly equidistant, anterior ocellus directed forward and situated slightly in advance of anterior margin of eyes, posterior ocelli directed laterally ; pigment bright red. Antennae about 2·2 times as long as head, structure normal to genus. Total length of antenna, 0·387 mm.

Prothorax about 1·35 times as wide as long, as wide at apex as at base, sides evenly rounded, slightly shorter than head and with similar reticulation ; colour distinctly darker than that of head. Pterothorax 1·5 times as wide as prothorax, deeply reticulated ; scutellum rugose, about 1·43 times as long as wide. Wings normal to genus. The scale of the fore pair heavily chitinized, and when the wings are at rest, fitting under the edge of the scutellum.

Abdomen about equal in width to pterothorax, sides deeply longitudinally rugoso-reticulate ; 10th segment divided above ; all bristles minute and scarcely visible. A dorsal pair near apex of the 10th segment more conspicuous than the others, slightly expanded and truncate at apex.

Male (*Macropterous*).—Colour and structure essentially as in female, except as follows : Prothorax brown, concolorous with head. Abdomen bright yellow, sides with blood-red hypodermal pigmentation ; sternites 3-7 each with a sharply delimited circular area at base ; 4th segment with a small, lateral, tooth-like projection ; segment 10 with the lateral bristles simple, not dilated in the form of a broad V.

Total length of antenna, 0·353 mm."

The following table gives measurements in mm. of nymphs and adults of *R. cruentatus* Hood. (Average of thirty measurements) :—

Stage	Length with caudal setae	Head		Eyes	Length of ante-nna	Prothorax		Pterothorax		Abdo-men	Greatest width
		Length	Width across eyes	Length		Length	Width	Length	Width	Length	
I . . .	0·774	0·101	0·107	0·025	0·264	0·085	0·135	0·220	0·210	0·368	0·196
II . . .	1·348	0·173	0·175	0·004	0·401	0·131	0·257	0·321	0·353	0·712	0·394
Prepupa . .	1·243	0·127	0·183	0·003	0·225	0·094	0·235	0·271	0·320	0·749	0·349
Pupa . .	1·507	0·168	0·196	0·017	...	0·146	0·281	0·288	0·329	0·904	0·349
Hood [1919]—											
Female . .	1·37	0·174	0·211	0·078	0·387	0·169	0·228	...	0·341	...	0·348
Male . .	1·08	0·166	0·203	0·072	0·353	0·152	0·208	...	0·300	...	0·293

IV. LIFE-HISTORY

Copulation.—The adults pair from two to ten days after emergence. When a female is ready for mating, it repeatedly moves the tip of its abdomen up and down, raises its wings and holds these in an upright position. The male at this stage crawls over the back of the female, and after every three to five seconds, rubs the tip of its abdomen over that of the female, which lifts its abdomen vertically up and so mating takes place. During copulation the male clasps the female with its middle legs. Copulation lasts for fifteen to forty-five minutes. When disturbed the pair at once separates. After mating both male and female begin feeding.

Two to seven days after copulation the male dies, while the female may live for as long as twenty days. Pre-oviposition period of a female varies from six to fourteen days (Table II).

Oviposition.—Eggs are laid singly, in slits made with the saw-like ovipositor, on the underside of a leaf. Excepting the end through which a nymph emerges, the egg lies buried in the leaf tissue. One to two minutes are required to deposit an egg. If a leaf containing eggs is held against a strong light, each egg appears as a very small bright spot.

Oviposition by the females of the first brood which emerge from over-wintered pupae starts about the second week of April. The succeeding broods continue to lay eggs up to November. The most active period for egg-laying, however, is June-July. When the rains set in, oviposition is greatly retarded.

The number of eggs laid by a single female in captivity varies from fifteen to fifty, the largest number being laid in July (Table I). The eggs are laid in instalments. During April-May and August-September, on an average, two eggs are deposited daily, while during June-July five to six eggs are laid every day.

TABLE I

Oviposition record of R. cruentatus *Hood on grape-vine leaves*

No.	Fertilized female sleeved		Total number of eggs laid
	From	To	
1	14·4	23·4	21
2	15·4	25·4	22
3	16·4	26·4	21
4	14·5	22·5	18
5	16·5	25·5	25
6	18·5	26·5	39
7	19·6	23·6	27
8	20·6	25·6	27
9	21·6	28·6	45
10	8·7	16·7	50
11	10·7	16·7	46
12	12·7	20·7	49
13	8·8	14·8	25
14	9·8	16·8	30
15	12·9	22·9	20
16	22·10	31·10	15

Hatching.—The duration of the egg stage varies with the season. The eggs laid in April-May and September-November hatch out in five to eight days (Table II), while those laid during June-August take three to four days to hatch. The egg about to hatch is recognised by two red spots—eyes of the nymph seen through the chorion—near its exposed end.

TABLE II

Pre-oviposition period and duration (in days) of egg-stage of R. cruentatus *Hood*

Adults emerged	Eggs laid	Pre-oviposition period	Eggs hatched	Duration of egg stage
25·3	8·4	14	16·4	8
3·5	10·5	7	15·5	5
4·6	10·6	6	13·6	3
19·7	27·7	8	31·7	4
15·8	23·8	8	27·8	4
15·9	1·10	14	6·10	5
23·10	3·11	11	9·11	6

Nymphal instars.—There are four immature instars, the third instar is known as the prepupa and the fourth as the pupa.

The duration of each instar varies according to the season (Table III).

TABLE III

Duration of nymphal instars of R. cruentatus *during the active period (For sake of brevity only 7 typical observations are given)*

	I	II	III	IV	V	VI	VII
First instar	16·4	13·5	19·6	13·7	15·8	26·9	25·10
	21·4	19·5	22·6	16·7	18·8	1·10	31·10
Days	5	6	3	3	3	5	6
Second instar	21·4	19·5	22·6	16·7	18·8	1·10	31·10
	27·4	23·5	25·6	19·7	21·8	6·10	6·11
Days	6	4	3	3	3	5	6
Prepupa	27·4	23·5	25·6	19·7	21·8	6·10	6·11
	5·5	27·5	29·6	22·7	24·8	13·10	14·11
Days	8	4	4	3	3	7	8
Pupa	5·5	28·5	29·6	22·7	24·8	13·10	14·11
	9·5	2·6	1·7	24·7	27·8	18·10	19·11
Days	4	5	2	2	3	5	5
Total duration	23	19	12	11	12	22	25

Thus the total duration of the immature stages varies from eleven to twenty-five days during April-May and September-November, and eleven to twelve days during June-August.

Pupation.—During the active period pupation takes place on the leaves. The pupa possesses power of locomotion and when disturbed it walks sluggishly and as if deliberately. During April-May and September-November the pupal stage lasts for four to five days, and during June-August it lasts for two to three days (Table III). Winter, however, is passed as a hibernating pupa in the soil, *Life-cycle.*—During the active period the total life-cycle is completed in fourteen to thirty-three days (Table IV). There are five to eight generations in a year.

TABLE IV

Duration of the life-cycle of R. cruentatus *(For sake of brevity only 9 typical observations are given)*

	I	II	III	IV	V	VI	VII	VIII	IX
Eggs laid . .	30·3	5·4	13·5	10·6	4·7	31·7	1·8	2·9	6·10
Adults emerged .	2·5	8·5	7·6	26·6	18·7	15·8	17·8	27·9	2·11
Total duration in days . . .	33	33	25	16	14	15	16	25	27

V. SEASONAL HISTORY

Usually the adults emerge from the hibernating pupae about the middle of March, but if the weather warms up earlier they may appear even in the beginning of March. Thus on the 3rd of March, 1932, a few adults—both males and females — were present on the old leaves of *Terminalia arjuna* and *Eugenia jambolana* at Lyallpur. From about the end of March to the end of October all stages of the pest are present in the field. During the first half of November, eggs, nymphs and pupae are commonly found. The adults mostly die off during November, although an occasional specimen may be obtained till the end. From about the middle of November to March only pupae are met with hibernating in the soil.

VI. PARTHENOGENESIS

Parthenogenesis is a common phenomenon among thrips, and is met with in *R. cruentatus* Hood, the parthenogenetically developed progeny consisting of males only. Sexual reproduction and parthenogenesis occur simultaneously and there are no alternation of generations. From the 5th to the 12th March 1932, a very large number of leaves from *Terminalia arjuna* and *Eugenia jambolana* were examined and the proportion of sexes was found to be 3·7 females to one male. Thus in the beginning of the season females preponderate and parthenogenesis occurs.

VII. HIBERNATION

As stated above, *R. cruentatus* Hood hibernates in the pupal stage, from the middle of November to March, in the soil under the host plant. Thus hibernation lasts for about four and a half months.

Depth suitable for hibernation

In order to find out the depth at which pupae hibernate under natural conditions, the following observations were carried out :—

In the months of January and February samples of soil measuring 6 in. by 6 in. up to a depth of 12 in. were taken from under : (*A*) heavily infested plants, (*B*) moderately infested plants, and (*C*) lightly infested plants. These soil samples were washed through very fine muslin cloth and the hibernating pupae separated (Table V).

TABLE V

Number of hibernating pupae at various depths in the soil

Depth (in inches)	Number of pupae obtained from the soil from under		
	(A)	(B)	(C)
1st	2	0	0
2nd	5	1	0
3rd	20	12	7
4th	37	21	11
5th	48	33	15
6th	28	17	7
7th	15	9	5
8th	9	6	3
9th	3	1	1
10th	1	0	0
11th	0	0	0
12th	0	0	0
Total	168	100	49
Dates of observation	2nd January 1930 to 26th January 1930	28th January 1930 to 10th February 1930	11th February 1930 to 22nd February 1930

It is seen from Table V that the hibernating pupae abound at depths of 3 in. to 7 in. with the largest number hibernating at a depth of 5 in. A few, however, may hibernate in the top 2 in. or may go down to a depth of 10 in. beyond which no hibernating pupae are found. Of the hibernating pupae sixty per cent. emerge as adults.

Soil compactness and hibernation

In order to study the effect of soil compactness on the penetration of the pupae into the soil for hibernation, the following observations were made :

Road dust was made to set firmly in a dish by the addition of water. When nearly dry, fifty thrips pupae were placed on its surface, and it was found that only nine pupae succeeded in making their way into the consolidated road dust to a depth of 2 in to 4 in. This, as well as similar other experiments, show that compactness of the soil is antagonistic to the successful hibernation of a vast majority of *R. cruentatus* Hood pupae which ultimately perish.

Soil texture and the emergence of adults from pupae

To see the effect of soil texture on the emergence of adults from the over-wintering pupae the following observations were made :—

(1) Soil was placed loosely in a dish and fifty pupae were placed on the surface. Out of this lot forty-two entered the soil and hibernated and of these twenty-six emerged as adults in the following March-April.

(2) In another dish with loose soil fifty pupae were placed and out of these forty-one hibernated. In January, and then again in February and March, just enough water was added to make the soil compact at the time of emergence. It was found that out of forty-one hibernated pupae only six emerged as adults.

From the above, as well as similar other experiments, it appeared that the emergence of adults from hibernating pupae was normal in the case of loose soil, but fell very considerably in compact soil.

VIII. PROGRESSION ON WATER

To test the effect of flooding on the survival of thrips, experiments were performed to determine their power of progression on water. For this purpose glass dishes of different diameters from 4 in. to 24 in. were taken (Table VI). In the centre of each dish a cardboard platform was erected. After filling the dish with water ten thrips were placed on the platform and their movements watched. All the thrips thus placed tried to escape by crawling. The results of these experiments are summarised in Table VI below.

TABLE VI

Power of progression of R. cruentatus *Hood adult on water (16th November 1929)*

Diameter of dish (in inches)	Time taken in crossing (minutes)	No. of thrips successful in crossing	Percentage
4	10	10	100
6	20	10	100
8	30	9	90
10	30	9	90
12	40	5	50
14	45	4	40
16	65	3	30
18	75	2	20
20	80	0	0
22	100	0	0
24	140	0	0

It was found that a female could crawl on water up to a distance of 9 in., while a male was quite exhausted after crawling a distance of 3 in. to 5 in.

IX. SUBMERGENCE OF ADULTS

It is interesting to note that thrips can stand submergence for a long period. Eight large glass dishes (each one foot in diameter) were filled with water to a depth of 3 in. In each dish fifty males and fifty females were made to sink down with needles. After every thirty minutes a dish was examined and the results tabulated as follows :—

TABLE VII

Submergence of R. cruentatus *Hood adults (15th November, 1929)*

Number of dish	Duration of submergence (minutes)	Number of survivals		Time taken to recover	Percentage mortality	
		Males	Females		Males	Females
1	30	50	50	Immediately . .	..	..
2	60	49	48	63 minutes .	2	4
3	90	47	47	125 ,, . .	6	6
4	120	29	30	265 ,, . .	42	40
5	150	10	12	640 ,, . .	80	86
6	180			All dead	100	100
7	210			,, ,,	100	100
8	240			,, ,,	100	100

Table VII shows that these thrips can stand ninety minutes' submergence successfully, but when it is prolonged beyond 150 minutes (*i.e.* 2½ hours), all of them die. This power of withstanding ninety minutes' submergence as well as the power of progression on water (Table VI) indicate that heavy rains accompanied or followed by strong winds do not affect *R. cruentatus* Hood adversely. Flooding of the vineyards as a means of control against *R. cruentatus* Hood is likely to be equally futile.

X. DISPERSAL

As is well known thrips do not possess satisfactory organs of flight, and for their dispersal they very largely depend upon the agency of wind, strong winds carrying them long distances. It has been observed that in absolutely calm atmosphere *R. cruentatus* Hood is only capable of leaping from one leaf to an adjacent one ; a female clearing at the most a distance of 12 in. and a male of 9·5 in. in one leap.

PROGRESS OF ATTACK

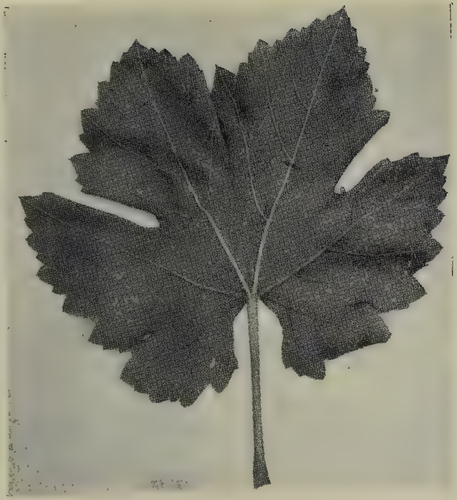


Fig. 1. Healthy grape-vine leaf.

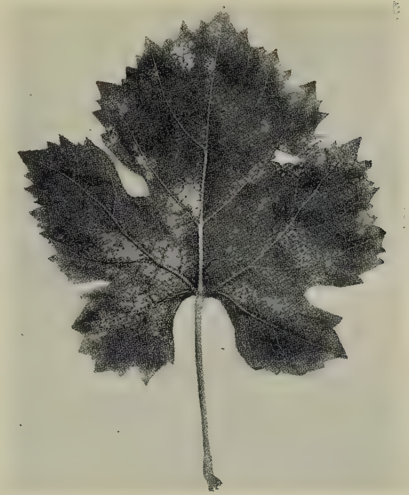


Fig. 2. Under side of grape-vine leaf with brown patches near midrib and lateral veins.

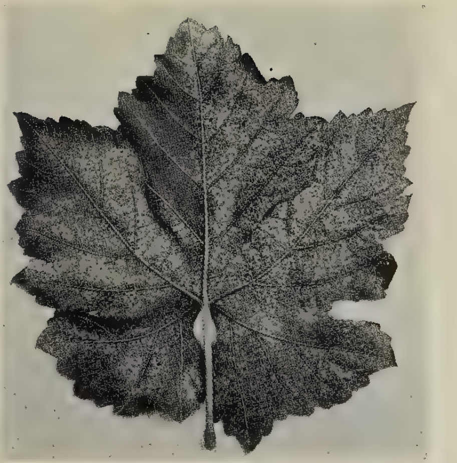


Fig. 3. Under side of grape-vine leaf with a few healthy patches.

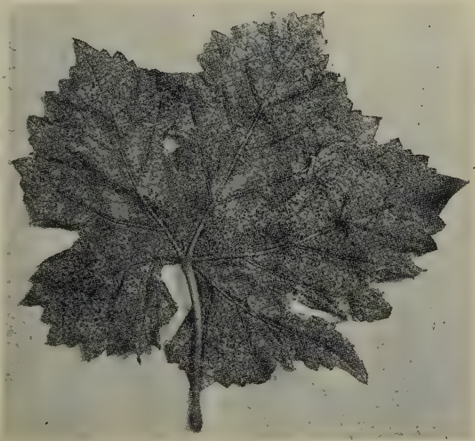


Fig. 4. Under side of grape-vine leaf entirely brown.

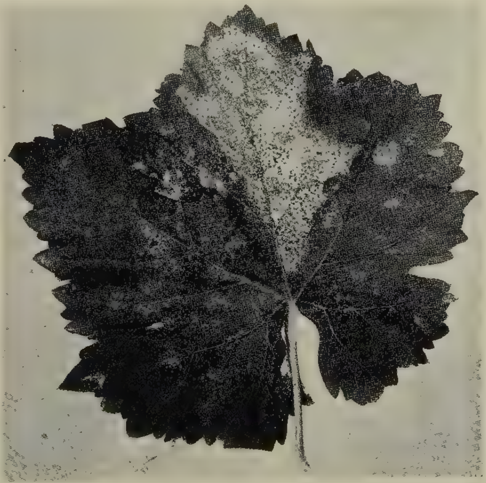


Fig. 5. Injury on the upper side of grape-vine leaf.

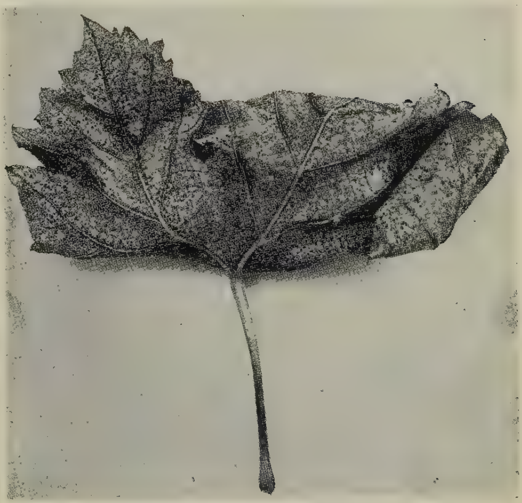


Fig. 6. Grape-vine leaf completely damaged and characteristically folded.

XI. Damage

Progress of attack

The thrips feed by rasping the leaf surface by their stylets and sucking the oozing cell sap. The injured surface is marked by a number of minute spots, thereby producing a speckled silvery effect which can be detected from a distance These spots eventually become brown.

Both adults and young do damage. They feed in groups, generally on the underside of a leaf : only in rare instances do they feed on the upper side.

In order to study the development of the symptoms of attack the following experiment was performed.

On the 27th of May, 1929, twenty thrips, adults as well as nymphs, were sleeved on a healthy and normal leaf (Plate LIV, fig. 1). By the 2nd of June 1929, the spots of injury were found in abundance. After sometime the spots ran together, at first forming large patches near the midrib and lateral veins (Plate LIV, fig. 2) and later on (by 14th-17th of June) spreading all over the leaf (Plate LIV, figs. 3, 4). At this stage the leaf assumed a light brown silvery colour and was covered with minute black spots– the excreta of the thrips. When the lower surface of the leaf was completely damaged the thrips began to attack the upper surface also (Plate LIV, fig. 5), until both the leaf surfaces were damaged and the leaf turned brown, withered, curled up, and ultimately fell off the plant on the 7th of July 1929 (Plate LIV, fig. 6). These are also the symptoms of attack under natural conditions. The leaves which were kept as control, however, did not show these signs but retained their usual healthy appearance.

It may be pointed out that when the attacked leaf begins to dry up, adults as well as nymphs migrate to healthy leaves. The thrips also attack the fruit, which develops a corky layer and becomes brown.

Extent of damage per thrips per day

In order to calculate the extent of injury done by *R. cruentatus* Hood per day, a known number of thrips were sleeved on healthy and normal leaves for a definite number of days. The damaged portion of the leaf was measured. As there was practically no difference between the average injury to leaves by adults and nymphs, therefore, all stages were sleeved together. The results of these observations are given in Table VIII.

TABLE VIII

Extent of damage done by R. cruentatus *Hood per day*

Number	Number of thrips per leaf	Duration of experiment (in days)	Damaged portion (sq. cm.)	Damage per thrips per day (sq. cm.)
1	10	44	41·90	0·0952
2	25	39	96·38	0·0988
3	10	30	28·56	0·0952
4	26	25	63·05	0·0970
5	25	25	61·13	0·0978
6	30	20	57·84	0·0964
7	27	20	53·94	0·0999
8	24	20	11·79	0·0983
9	23	20	43·97	0·0956
10	22	20	43·34	0·0985
11	21	20	37·64	0·0920
12	29	19	52·07	0·0945
13	28	19	51·44	0·0967
14	20	19	35·42	0·0932
15	25	18	38·72	0·0968
16	14	18	24·82	0·0985
17	5	17	8·12	0·0954
18	20	16	29·45	0·0920
19	15	15	21·98	0·0977
20	15	14	20·96	0·0993

Thus the average injury caused by a thrips in a day is 0·0964 sq. cm., and if there are, on an average, twenty-five thrips per leaf, the attack may be considered as serious.

Intrinsicality of damage

To study the damage done to the cells, injured leaves were sectioned. The adults as well as nymphs first attack the lower epidermal layer round about the stomata. The first step in this injury is the destruction of a part of the surface of the lower epidermis and the spongy tissue of the leaf (Plate LV, figs. 2, 3). The injury proceeds still further, till at the final stage all the cells of the spongy parenchyma as well as the pallisade parenchyma are destroyed (Plate LV, fig. 4). Obviously such a leaf cannot perform its functions properly.

Effect of attack on yield

It is evident from a record of two years' yield of grapes in the Botanical Experimental Garden, Lyallpur, that the varieties which resist the attack of these thrips yield better than the susceptible varieties. Of the susceptible varieties, the sprayed plants yield more than the untreated plants (Table IX). The fruit obtained from seriously attacked plants is of poor quality and fetches low price.

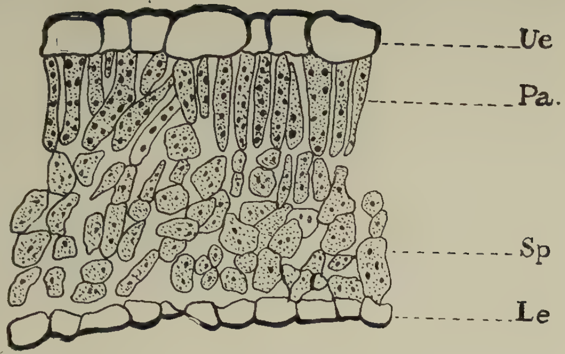


FIG. 1. Section of healthy grape-vine leaf.

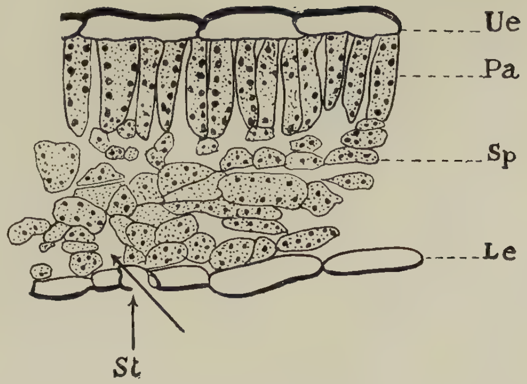


FIG. 2. Section of grape-vine leaf showing commencement of injury (indicated by the longer arrow).

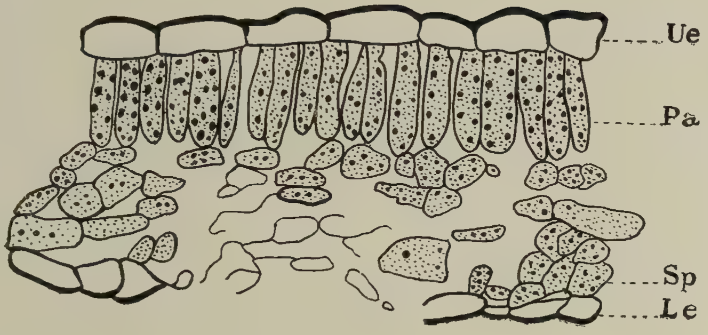


FIG. 3. Section of grape-vine leaf showing injury fairly well advanced.

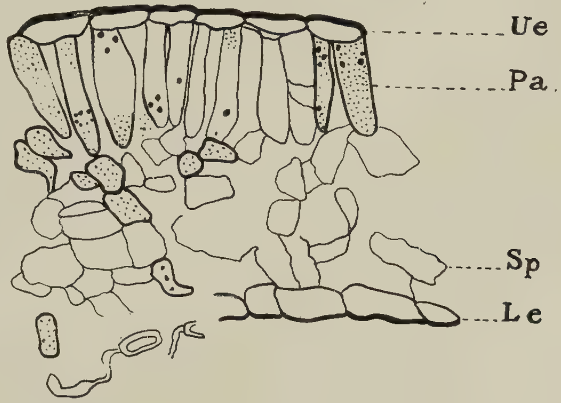


FIG. 4. Section of grape-vine leaf showing final stage of injury.

Ue.—Upper epidermal layer. *Le.*—Lower epidermal layer.
Pa.—Pallisade tissue. *Sp.*—Spongy tissue. *St.*—Stomata.

TABLE IX

Effect of R. cruentatus *Hood attack on the yield of grapes*
(*Yield from 12 plants*)

	Untreated	Treated	Resistant
	lbs. oz.	lbs. oz.	lbs. oz.
1929—			
	25 10	57 0	164 14
	22 0	56 14	91 4
1930—			
	29 0	59 14	160 10
	25 14	58 10	93 12

XII. SUSCEPTIBILITY OF DIFFERENT VARIETIES OF GRAPE-VINE

Among the varieties of grapes grown at the Experimental Fruit Farm, Agricultural College, Lyallpur, the *Jaishi*, which has thick leaves with hairy underside, is the only variety which remains free from the attack of this thrips. In fact such varieties as *Tur*, *Tas*, *Bedana*, *Sursavi*, *Kishmish* planted near the *Jaishi* variety, were badly attacked and yet the *Jaishi* remained free. Adults as well as nymphs were repeatedly sleeved in large numbers on the *Jaishi*, but in none of the experiments *R. cruentatus* could be bred on this variety, the insects sleeved dying within four days.

XIII. RESISTANCE OF *R. cruentatus* HOOD TO COLD

The experiments to test the resistance of adults of *R. cruentatus* Hood to cold were carried out during May 1929, in a kelvinator. In each experiment twenty-five males and females were subjected to a temperature of 4°C. The results of these experiments are given in Table IX below.

TABLE IX

Resistance of R. cruentatus *Hood to cold*

Serial number	Time for which exposed (minutes)	Condition after exposure	Time taken to recover (minutes)	Remarks
1	5	All active . . .	. .	
2	10	6 quiescent . . .	1	
3	15	All ,, . . .	1—3	
4	20	,, . . .	2—5	
5	25	,, . . .	2—7	
6	30	,, . . .	4—9	
7	35	,, . . .	4—9	
8	40	,, . . .	4—10	
9	45	,, . . .	4—13	

TABLE IX—*contd.*

Serial number	Time for which exposed (minutes)	Conditions after exposure	Time taken to recover (minutes)	Remarks
10	50	All quiescent . .	7—19	
11	55	,, . . .	10—24	
12	60	,, . . .	15—27	
13	90	20 quiescent (rest dead)	15—30	5 males dead.
14	120	18 ,, ,, . .	19—45	6 ,, 1 female } ,,
15	150	15 ,, ,, . .	15—40	7 males 3 females } ,,
16	180	13 ,, ,, . .	20—42	8 males 4 females } ,,
17	210	10 ,, ,, . .	25—50	11 males 4 females } ,,
18	240	5 ,, ,, . .	30—55	14 males 6 females } ,,
19	270	1 ,, ,, . .	50—59	14 males 10 females } ,
20	300	All dead . . .	..	
21	330	,, . . .	..	
22	360	,, . . .	..	

It will be seen from the above table that the adults can stand exposure to 4°C. for one hour, all of them becoming active fifteen to twenty-seven minutes after their removal from the kelvinator. When exposed for ninety minutes twenty per cent of them however die, the death-rate increasing with longer exposures until at an exposure of 300 minutes or more all of them succumb. It will also be noticed that the males are comparatively less resistant to cold than the females.

XIV. PARASITE

The specimens of an endoparasite collected by Mr. M. Afzal Husain were sent to the Imperial Institute of Entomology, London for identification. Waterston [1930] named the parasite as *Thripoctenus maculatus* sp. Nov. (Eulophidae : Hymenoptera), and his description is reproduced below :—

" *Male and female.*—Head and thorax shining black, the former with cupreous, the latter with violaceous or deep blue metallic reflections. Coxae of fore legs black. Wings hyaline ; sutures of head, antennae, and legs pale. Abdomen nearly white with some blackish spots, mainly at sides, behind one half in female on segments V-VI, in male on segments IV-V.

Female.—Length 0·8–0·9 mm. ; expanse about 1·4 mm.

Male.—Length 0·7–0·75 mm. ; expanse about 1·4 mm.

Female.—Head just wider than thorax. Antennae about 0·33 mm. in length. Scape with bulla a little longer than pedicel, ring-joint and two funicular joints. The latter short and slightly transverse. Second suture of club indistinct.

The antennae in both the sexes are extremely alike, but in the male the scape is a little wider and shorter in proportion to the rest of the antenna, and the large dorsal sensorium on the first segment of the club appears to be absent ".

Description of pupa.—Length 0·92–1·01 mm. ; width across the 'shoulders' 0·39–0·43 mm. Cylindrical. Deep red when newly formed, becoming piceous before emergence. Head oval, prominent in front.

Life-history and habits.—The adults copulate on the vine leaves. The number of eggs laid per female varies from 30 to 105. Three to five days after mating a fertilized female searches out a first or second instar thrips nymph, taps its abdomen, pierces it with its long, thin, and slender ovipositor, and lays an egg therein.

Eggs hatch out in five to ten days, and the larval stage lasts for three to six days. When ready to pupate, the parasite ruptures the skin of the host at the head. Pupation always takes place in front of the host's skin, which often remains attached to it. The pupal stage lasts for fifteen to twenty-nine days. The adult escapes from the pupal skin by splitting it along its mid-dorsal line. Thus the life-cycle is completed in twenty-three to forty-five days.

Hibernation.—The hibernation period of the parasite synchronizes with that of the host, the parasite hibernating as a pupa on the host plants of *R. cruentatus* from November-December to March-April.

Recognition of the parasitized nymph.—The newly parasitized nymph has its antennae, legs and anal portion of abdomen white, and middle of abdomen deep pinkish. It moves about freely and feeds normally. As the parasite grows older the sides of the body of the parasitized nymph become white, and a deep red spot develops in the centre of its abdomen. At this stage its movements become laboured, it stops feeding and ultimately dies. After death its body becomes rounded and swollen at first but ultimately assumes a cylindrical form with the antennae and legs reduced to mere rudiments.

Parasite population.—The parasite population is very high towards the end of the season (Table X).

TABLE X

Parasite population in November 1929

Date of examination	Number of leaves examined	Average No. of parasite pupae per leaf
10th November	25	285
11th November	20	141
12th November	20	24
13th November	30	340
14th November	40	310
15th November	10	98
16th November	25	25
17th November	20	128
18th November	30	82

XV. PREVENTIVE MEASURES

1. *Agricultural operations*

As stated above *R. cruentatus* hibernates in the pupal stage in the soil at a depth of 3 in. to 7in. These hibernating pupae are the source of re-infestation next spring. Root opening is a common practice with the vine growers and this operation exposes the pupae to adverse conditions. If the soil removed from under the grape-vines is carted away and replaced by fresh soil free from pupae, the incidence of attack in the following season is greatly lessened.

2. *Sticky bands*

The adults emerging from the overwintered pupae crawl up the stem of their food-plants. They have been caught on sticky bands placed round the stems of *Eugenia jambolana* and *Terminalia arjuna*. On eight such bands 104 thrips (an average of 13 per band) were caught between the 8th and 15th of March 1932. On grape-vines, however, these sticky bands did not yield positive results.

3. *Baiting*

Various chemicals recommended by Howlet [1914] as baits for thrips were tried. These baits were kept exposed from 14th to 20th June, 1930 (the period of greatest activity), and observations taken twice daily. The results obtained were very discouraging, the maximum number of thrips attracted was five (attracted by salicyaldehyde-benzalaldehyde-formalin ; salicyaldehyde-benzal-nitrobenzene-formalin). The baits were, therefore, given up as useless.

XVI. CONTROL MEASURES

1. *Biological control*

Leaves with the parasitised nymphs of *R. cruentatus* should be collected and kept in proper cages so that the parasite on emergence are able to escape, and carry on the destruction of the pest.

2. *Insecticidal control*

R. cruentatus Hood can be easily destroyed by spraying with contact insecticides. The following have been tried successfully :—

(*a*) *Tobacco decoction.*—Half a seer of tobacco refuse (stem, leaves, dust, etc.), was soaked in one gallon of water for twenty-four hours, and then strained. To the strained liquid was added two *chhataks* of cheap soap cut up into thin slices and the solution stirred vigorously to dissolve the soap thoroughly. This stock solution diluted with four parts of water gave ninety-seven to ninety-eight per cent mortality (Table XI). The adults died at even a weaker strength, but the prepupae and the pupae were more resistant.

Without soap, tobacco decoction gave very unsatisfactory results, for it killed only twenty to thirty per cent of the thrips.

(*b*) Ak (Calotropis procera) *decoction.*—Fresh leaves, twigs, and flowers of *ak* were chopped up, and boiled in water for two hours. After straining the liquid, cheap soap cut into small pieces was added, and the liquid stirred vigorously to dissolve the soap thoroughly. The decoction was prepared according to the following formula :—

Ak leaves, etc.	1 seer
Cheap soap	2 *chhataks*
Water	1½ gallons

This gave eighty-six per cent mortality (Table XI), and the sprayed thrips took five to ten minutes to die. Without soap this material also gave very poor results, mortality never exceeding twenty-five per cent.

Ak is very common throughout India, and is thus easily obtainable. Its extensive use as an insecticide will not only control the grape-vine thrips, but will also insure the eradication of this otherwise troublesome weed.

(*c*) Nim (Melia azadirichta) *decoction.*—The same formula was used as in the preceding case, the results obtained were very unsatisfactory—average mortality being ten to fifteen per cent. Without soap *nim* decoction was absolutely ineffective.

(*d*) *Black leaf 40.*—This preparation gave a mortality of ninety-seven per cent (Table XI) when used according to the following formula :

Black leaf 40	¼ seer
Cheap soap	½ seer
Water	100 gallons

TABLE XI

Efficiency of different insecticides against R. cruentatus *Hood. For population twenty leaves per plant were selected at random. Cost of spraying per plant varied from Re. 0-0-1 to Re. 0-0-5.*

(Tas *variety of grape-vine was selected for the tests*)

Name of poison	Date of spraying	No. of living thrips (all stages) before spraying			No. of dead thrips after spraying			Per cent mortality
		Minimum per leaf	Maximum per leaf	Total	Minimum per leaf	Maximum per leaf	Total	
Tobacco decoction	28th May 1930 . .	6	40	374	1	2	9	97·9
Do. .	Do. .	6	44	454	1	2	9	98·3
Ak decoction .	24th April 1930 . .	10	201	2,021	2	31	276	86·5
Do. .	Do. .	55	180	1,982	6	34	276	86·5
Black leaf 40 .	6th November 1929 .	3	20	287	..	1	6	97·9
Do. .	Do. .	3	24	177	..	1	5	97·7

Soft soap when used alone at the rate of three *chhatanks* in four gallons of water gave twenty to thirty per cent mortality.

Successful spraying.—To be effective, spraying should be done on calm and fine days. The machine employed should produce a fine misty spray. The amount of insecticide required per plant will vary with its size, but, on an average, 1½ seers are quite sufficient per average-sized plant. During spraying the underside of the leaves, where the pest abounds, must receive special attention.

3. *Fumigation*

It may be mentioned that fumigation with hydrocyanic acid gave cent per cent mortality, but was more expensive.

XVII. ACKNOWLEDGMENTS

The problem was suggested by Mr. M. Afzal Husain, Entomologist to Government, Punjab, Lyallpur to whom we are grateful for guidance during the progress of the work, and for help in writing up this paper which was completed in the Zoological Laboratory, Cambridge, where the senior author worked from October, 1934 to June, 1936. We are indebted to Dr. A. D. Imms, F. R. S., for going through the manuscript and suggesting useful alterations.

XVIII. Summary

Rhipiphorothrips cruentatus Hood has been found on grape-vine leaves and a number of other host plants in the Punjab and South India.

Descriptions of the egg, nymphal, pupal, and adult stages are given.

Life-history is described in detail. During the active period the life-cycle is completed in fourteen to thirty-three days.

The pest hibernates in the pupal stage in the soil from mid-November to March in the top 3 in.-7 in. Adults emerging from these pupae start infestation in the following spring. Compact soil interferes with pupal hibernation and emergence of adults.

Parthenogenesis, dispersal, progression on water, and submergence of adults are discussed.

Both adults and young do damage which is described in detail. Yield from the attacked plants is considerably lowered, and the fruit obtained is of low quality, and fetches low price.

Jaishi variety is immune to the attack of this thrips.

Thripoctenus maculatus Watrstn.—an important endo-parasite—is described in detail.

Root opening followed by the replacement of dug-out soil by fresh soil is a good preventive measure.

The pest can be effectively and economically controlled by spraying with tobacco decoction, *ak* decoction, or black leaf 40.

Tent fumigation with hydrocyanic acid is also very effective, but is expensive.

REFERENCES

89 Afzal Husain, M. (1923). *Report Dept. Agric. Punjab*, Pt. II.
262 Hood, J. D. (1919). *Ins. Insc. Men.* **7**, 94.
271 Howlet, F. M. (1914). *Jour. Econ. Biol.* **9**, 21.
101 Karny, H. H. (1927). *Mem. Dept. Agric. Ind.* (*Ent. Ser.*) **9**, 187.
17 19 Ramakrishna Ayyar, T. V. (1929, 1). *Mem. Dept. Agric. Ind.* (*Ent. Ser.*) **10**, 217.
16 48 ——————. (1929, 2). *Bull. Ent. Res.* **20**, 77.
26 ——————. (1932). *Agric. Live-stock Ind.* **2**, 391.
✓ Waterston, J. (1930). *Ann. Mag. Nat. Hist. Ser.* **10**, 5 : 243.

GERMINATION OF FUNGAL SPORES IN RELATION TO ATMOSPHERIC HUMIDITY

BY

S. CHOWDHURY
Imperial Agricultural Research Institute, New Delhi

(Received for publication on 4th December 1936)

CONTENTS

PAGE
I. INTRODUCTION 653
II. MATERIAL AND METHODS 654
III. RESULTS 655
IV. SUMMARY 657
REFERENCES 657

I. INTRODUCTION

ONE of the most essential factors for the germination of spores of fungi is water. According to several investigators the humidity necessary for any spore germination whatever must be close to saturation, apparently ninety-five per cent or higher, *e.g. Puccinia coronifera* Kleb. (*P. coronata* Cda.) [Fromme, 1913], *Colletotrichum lindemuthianum* (Sacc. and Magn.) Bri. and Cav., *Puccinia graminis* Pers. var. *tritici* Erikss. and Henn. and *Ascochyta fagopyrum* Thuem et Boll [Lauritzen, 1919], *Puccinia graminis* Pers. [Melhus, Durrell and Kirby, 1920]. Spores of some species apparently must be in contact with water before they will germinate; *e.g.* teleutospores of *Gymnosporangium juniperi-virginianae* Schw. [Weimer, 1917], uredospores of *Puccinia coronata* Cda. [Melhus and Durrell, 1919] and aecidiospores and uredospores of *Melampsora lini* (Pers.) Lév. [Hart, 1926]. On the other hand, uredospores of *Puccinia graminis* Pers. var. *tritici* Erikss. and Henn. [Lauritzen, 1919] and aecidiospores of *Gymnosporangium claviceps* (C. & P.) (*G. germinale* Kern.) [Doran, 1922] do not require condensed moisture for spore germination.

Lesage [1895] was the first to determine accurately the humidity requirement for the germination of fungus spores. To obtain atmospheres containing different percentages of moisture he used sodium chloride solutions of different concentrations. He worked with spores of *Penicillium glaucum* and found that the minimum degree of moisture required for spore germination was between eighty-two and eighty-four per cent relative humidity. Krakover [1917] determined the minimum relative humidity required for the germination of spores of *Macrosporium sarciniforme* Cav. and found it to be between 92·8 and 93·4 per cent relative humidity. According to Smith [1926] only three per cent of the uredospores of *Puccinia Sorghi* Schw. (*P. maydis* Béreng) germinated at 97·5 per cent relative humidity. Newhall [1928] observed that germination of the spores of *Cladosporium fulvum* Cke. is inhibited in an atmosphere having a relative humidity below ninety-six per cent at ordinary temperatures. Tomkins [1929] examined the rate of germination of certain mould spores and found that the minimum relative humidity for growth was eighty-five to ninety per cent for most of the species tested. According to Hemmi and Abe [1933] uredospores of *Puccinia triticina* Erikss., *P. glumarum* (Schm.) Erikss. and Henn., and *P. lolii* Niels (*P. coronata* Cda.) could not germinate at or below ninety-five per cent relative humidity. Abe [1933] showed that the spores of *Piricularia oryzae* B. et C. could germinate, though slightly, at ninety-six per cent relative humidity but not at all at ninety-two per cent. Groom and Panisset [1933] quote a very low minimum for the spores of *Penicillium chrysogenum* Thom—eighty-one per cent on glass and seventy-three per cent relative humidity on book-binding material. For spores of *Helminthosporium nodulosum* Berk. and Curt., Mitra and Mehta [1934] cite ninety-one per cent relative humidity as the lowest limit for spore germination. Galloway [1935] determined the minimum moisture requirements for the germination of spores of about forty fungi. He found that the spores of certain types of *Aspergillus* and *Penicillium* could germinate at relative humidities as low as seventy-five per cent although most required eighty to eighty-five per cent; species of *Cladosporium*, *Thielaviopsis*, *Alternaria* and *Acrothecium* required humidities of ninety per cent or slightly more. Chowdhury [1936] gives ninety-five per cent as the minimum relative humidity for the germination of the spores of *Colletotrichum graminicolum* (Ces.) Wils.

In view of the importance of spore germination in relation to infection, and since water is one of the most essential factors for germination of fungus spores, it is of great importance in the study of any fungus disease to know the least amount of moisture necessary for the germination of its spores. In the present paper the germination of spores of certain Indian fungi in relation to relative humidity is recorded.

II. MATERIALS AND METHODS

The following organisms were employed in the present investigation :—

(1) *Acrothecium penniseti* Mitra

(2) *Alternaria brassicae* (Berk.) Bolle

(3) *Helminthosporium frumentacei* Mitra

(4) *Cladosporium herbarum* (Pers.) Link

(5) *Colletotrichum falcatum* Went

(6) *C. lindemuthianum* (Sacc. and Magn.) Br. and Cav.

(7) *Gloeosporium tabernaemontanae* Speg.

(8) *Phyllosticta cajani* Syd.

Since it is essential that the substratum should be in equilibrium with required humidity at the time an experiment is begun, and not still drying up or taking up water, the minimum amount of moisture required for the germination of the spores of fungi used in this investigation was determined by the method suggested by Galloway [1935]. Thin sheet viscose (*e.g.*, ' cellophane ', but not the moisture proof variety) was used as a substratum. The viscose sheet was soaked in one per cent glucose (Merck) solution and after blotting off the excess liquid it was dried in the air. The viscose was then cut into half-inch squares.

The spores to be examined were placed on these half-inch viscose squares in the form of a thin streak; this was most conveniently done, as suggested in the paper quoted above, by folding the squares in half and lightly touching with the folded edge the sporing surface of the medium. In the case of *Phyllosticta cajani* Syd. the pycnidia were crushed on the viscose sheets and then dried in desiccators. These viscose sheets with the spores on them were then suspended by loops of thread in stoppered wide-mouthed bottles of about 500 c.c. capacity, each containing 30 c.c. of a suitable humidity solution—sodium chloride solutions [Lesage, 1895] and sulphuric acid solutions [Stevens, 1916] of definite specific gravity were used. The bottles were then kept at 25°C. For each relative humidity test a large number of half-inch squares of viscose sheet with the spores were used. At intervals these pieces were removed, fixed in lacto-phenol and examined microscopically.

III. Results

Tomkins [1932] suggests that three criteria should be employed in studies of spore germination; (i) the latent period of germination, (ii) the percentage germination and (iii) the rate of elongation of the germ tubes. In this study, however, only the percentage of spore germination at intervals was determined. Attempts were made to determine the average length of the germ tube at intervals but this was found practically impossible owing to the rapid elongation and overlapping of the germ tubes. Where moisture requirements are the only factor under test the rate of elongation of the germ tubes varies with latent period of germination.

The results are recorded in Table I and are based on the counting of 200 spores at random.

TABLE I

Percentage germination at different relative humidities

Fungus	Hours	Relative humidity percentage								
		100	96·9	95·0	93·9	92·7	91·0	90·0	87·5	84·9
Acrothecium penniseti	5	34·5	24·7	17·6	9·0	6·2	..	..	..	..
	20	82·6	64·7	39·8	21·6	15·9	8·7	2·0	..	..
	45	95·2	79·5	52·4	34·2	24·6	17·7	7·9	..	..
	78	98·0	83·7	57·6	39·4	29·0	20·1	9·0	..	..
Alternaria brassicae	6	36·5	27·0	19·6	13·8	9·8	..	..	..	..
	22	88·5	67·6	42·9	23·2	16·7	5·2	2·1	..	..
	48	96·1	82·7	61·6	32·0	22·6	12·1	7·6	..	..
	76	98·7	88·1	66·9	36·4	25·0	18·6	10·7	..	..
Helminthosporium frumentacei	6	37·5	28·0	20·6	14·8	8·8	..	..	..	..
	20	88·7	68·8	47·2	26·1	17·7	6·0	..	..	..
	48	96·2	83·7	59·6	34·2	23·1	9·7	..	..	..
	78	98·0	86·9	63·1	38·2	26·1	11·8	..	..	..
Cladosporium herbarum	5	27·6	18·0	10·4	4·8	2·0	..	..	..	..
	20	78·6	57·8	32·1	15·2	9·4	4·6	1·0	..	..
	42	89·1	78·3	50·4	24·3	13·7	8·2	5·3	..	..
	70	94·6	82·5	54·3	27·0	15·0	9·6	6·7	..	..
Colletotrichum falcatum	16	30·5	18·3	..	..	..	..	..	..	..
	40	76·3	45·0	8·7	..	..	..	..	..	..
	80	90·5	68·7	21·4	..	..	..	..	..	..
	124	92·0	75·6	24·1	..	..	..	..	..	..
C. lindemuthianum	12	24·6	12·2	..	..	..	..	..	..	..
	24	57·0	36·2	4·3	..	..	..	..	..	..
	48	88·7	52·4	10·9	..	..	..	..	..	..
	96	92·6	56·7	12·0	..	..	..	..	..	..
Gloeosporium tabernaemontanae	18	36·8	16·7	7·2	..	..	..	..	..	..
	40	82·4	47·6	27·9	5·2	..	..	..	..	..
	78	91·4	67·6	45·0	11·5	..	..	..	..	..
	120	94·0	72·4	51·2	14·0	..	..	..	..	..
Phyllosticta cajani	12	32·0	23·5	..	..	..	..	..	..	..
	24	66·2	45·9	6·2	1·0	..	..	..	..	..
	48	87·2	61·9	15·7	4·0	..	..	..	..	..
	107	91·2	66·7	18·0	6·2	..	..	..	..	..

The results recorded in Table I show that the time required for spore germination is the least and the percentage of germination greatest at 100 per cent relative humidity for the spores of all species of fungi tested. The time required for germination increases and the percentage germination decreases with the lowering of the relative humidities till the minimum is reached. None of the fungi tried germinated below ninety per cent relative humidity. This lies between the figures for the purely parasitic rusts and the purely saprophytic moulds such as *Aspergillus* and *Penicillium*. Moreover the four fungi germinating below 93·9 per cent relative humidity are genera often saprophytic on food, fabrics, etc., the remainder being rarely found except on plant tissues.

IV. SUMMARY

Experiments were conducted to determine the relation of atmospheric humidity to the germination of spores of *Acrothecium penniseti* Mitra, *Alternaria brassicae* (Berk.) Bolle, *Helminthosporium frumentacei* Mitra, *Cladosporium herbarum* (Pers.) Link, *Colletotrichum falcatum* Went, *C. lindemuthianum* (Sacc. and Magn.) Br. and Cav., *Gloeosporium tabernaemontanae* Speg. and *Phyllosticta cajani* Syd. The minimum relative humidity for spore germination was found to be ninety per cent for some while others did not germinate below ninety-five per cent relative humidity.

REFERENCES

Abe, T. (1933). *Forsch. auf dem Geb. der Pflanzenkrankh (Kyoto)* **2**, 98-124.

Chowdhury, S. C. (1936). *Ind. Jour. Agric. Sci.* **6**, 833-843.

Doran, W. L. (1922). *Bull. Torr. Bot. Club* **49**, 313-340.

Fromme, F. D. (1913). *Bull. Torr. Bot. Club.* **40**, 501-521.

Galloway, L. D. (1935). *Jour. Text. Inst.* **26**, T 123-T 129.

Groom, P. and Panisset, T. (1933). *Ann. Appl. Biol.* **20**, 633-660.

Hart, H. (1926). *Phytopath.* **16**, 185-205.

Hemmi, T. and Abe, T. (1933). *Forsch. auf dem Geb. der Pflanzenkrankh (Kyoto)* **2**, 1-9.

Krakover, L. J. (1917). *19th Ann. Rept. Mich. Acad. Sci.* 275-327.

Lauritzen, J. I. (1919). *Phytopath.* **9**, 7-35.

Lesage, P. (1895). *Ann. Sci. Nat. Bot.* **8**, 309-322.

Melhus, I. E. and Durrell, L. W. (1919). *Res. Bull. Iowa Agric. Expt. Stn.* **49**.

Melhus, I. E.; Durrell, L. W. and Kirby, R. S. (1920) *Res. Bull. Iowa Agric. Expt. Stn.* **57**.

Mitra, M. and Mehta, P. R. (1934). *Ind. Jour. Agric. Sci.* **4**, 943-975.

Newhall, A. G. (1928). *Bimonthly Bull. Ohio Agric. Expt. Stn.* **13**, 119-122.

Smith, M. A. (1926). *Phytopath.* **16**, 69.

Stevens, N. E. (1916). *Phytopath.* **6**, 428-432.

Tomkins, R. G. (1929). *Proc. Roy. Soc.* **B. 105**, 375-401.

————— (1932). *Trans. Brit. Myc. Soc.* **17**, 147-149.

Weimer, J. L. (1917). *Bull. Cornell Agric. Expt. Stn.* **390**.

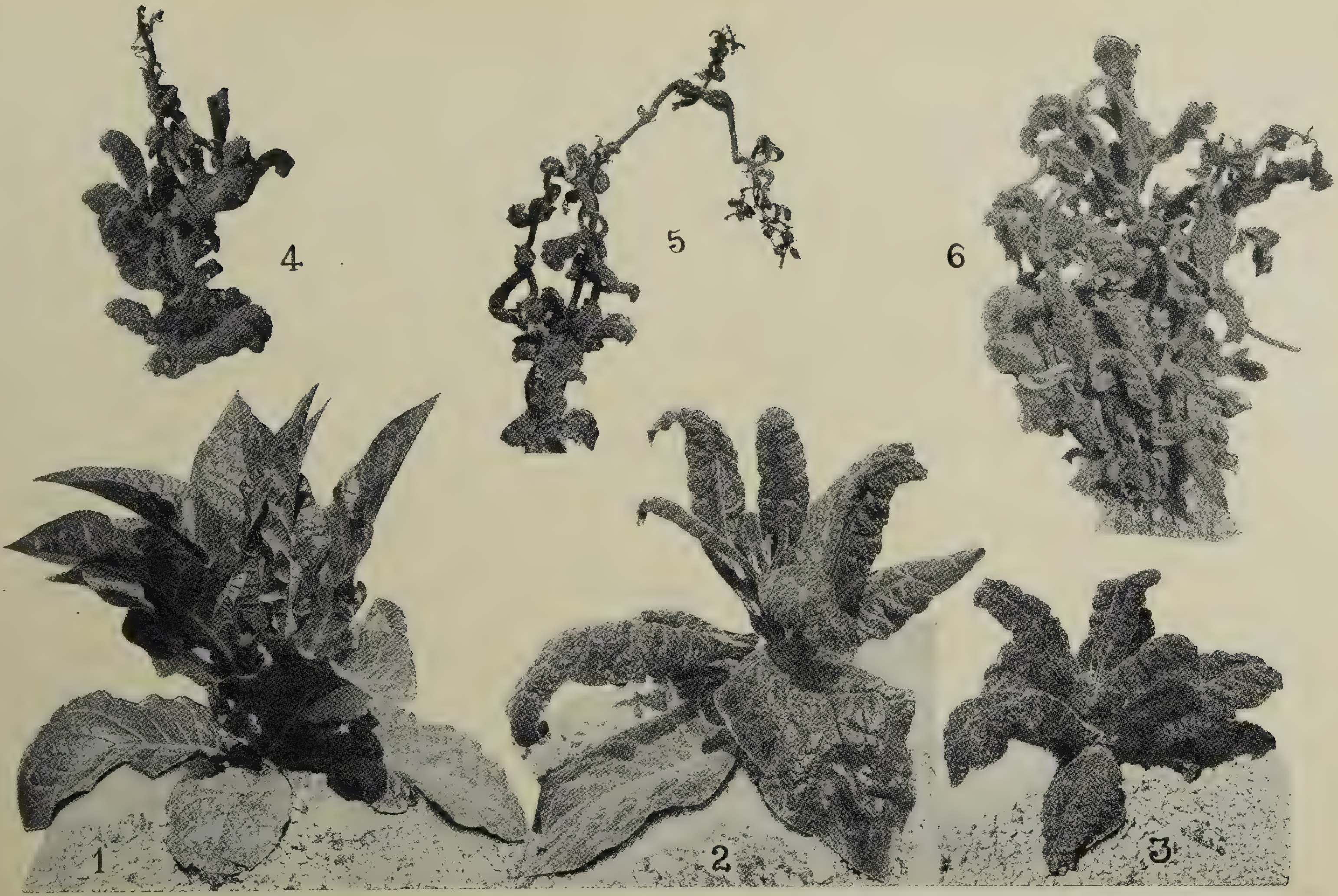


FIG. 1. A healthy tobacco plant (photo.)

FIGS. 2-6. Tobacco plants suffering from various types of leaf-curl disease (photo).

ENTOMOLOGICAL INVESTIGATIONS ON THE LEAF-CURL DISEASE OF TOBACCO IN NORTH BIHAR

A. M. L

I. TRANSMISSION EXPERIMENTS WITH SOME SUSPECTED INSECT VECTORS

II. AN ALTERNATIVE HOST OF THE VIRUS AND THE INSECT TRANSMITTER

BY
HEM SINGH PRUTHI, M.Sc., Ph.D. (Cantab.), F. N. I.
Imperial Entomologist
AND
C. K. SAMUEL, B.Sc.
Assistant to the Imperial Entomologist, Imperial Agricultural Research Institute, New Delhi

(Received for publication on 11th November, 1936.)

(With Plates LVI-LVIII)

Introductory

The leaf-curl disease of tobacco is very common at Pusa and several other localities in North Bihar. During the last three years the incidence of this disease in the various plots of tobacco at Pusa has been as high as fifty to seventy per cent. The affected plants are often very much stunted in growth, the stem being sometimes badly twisted, and their leaves are small, curled, twisted, thickened and puckered (Plates LVI and LVII). Not only the vegetative organs but the flowers and the seed pods of the affected plants also show distinct effect of the disease. Their flowers (variety H 142) are of dull reddish colour and the pods are thick and rough looking, whereas the healthy plants have bright pink flowers and smooth pods. The value of the tobacco crop being entirely dependent on the texture and size of the leaves, it is easy to imagine the amount of loss which is caused by the leaf-curl disease every year.

There are several types of leaf-curl at Pusa and our colleague, Dr. B. P. Pal, Second Economic Botanist, has carefully studied the symptoms of the various types and some other botanical aspects of the disease. The reader is referred to his paper on the subject published in this *Journal* (Vol. 7, pp. 363-93).

The entomological investigations on the leaf-curl disease of tobacco were started at Pusa soon after the senior author joined the Imperial Institute of Agricultural Research in September 1934. Naturally the first item on the programme of study was the investigation of the exact nature of the insect fauna which is found on this crop throughout its growth. For this purpose collections of insects on tobacco have been made at different times of the day from the beginning of October up to the end of March during the two seasons 1934-35 and 1935-36. The collection is very rich and interesting and its detailed account will be published in a separate communication. The most common insect vectors of virus diseases of plants are known to be sucking insects [Kunkel, 1926; Storey, 1931, 1932; Smith, 1933; Severin, 1934, etc.]. Insects of this kind which have been most commonly met with on tobacco at Pusa are the capsid bug, *Engytatus tenuis* Reut. (=*Gallobelicus crossicornis* Dist.), * the white-fly, *Bemisia gossypiperda* M. & L. and the aphid *Macrosiphum* sp. Of these *E. tenuis* was found breeding in large numbers on tobacco almost throughout the season, whereas the latter two occurred in comparatively fewer numbers on this crop. The white-fly was obtained on tobacco in the adult stage only, while the aphid was found in adult (alate) up to the beginning of February after which it began to breed on this crop. In view of these observations *E. tenuis* was suspected to be a probable vector of the disease and therefore was the first to be selected for transmission experiments, which are described below.

I. TRANSMISSION EXPERIMENTS WITH *Engytatus tenuis* REUT.

Species of *Engytatus* are of common occurrence on tobacco in several parts of the world. The Indian species *E. tenuis* is found in the East Indies, Formosa, etc. [Esaki, 1926; Thung, 1932], whereas two other species of this genus are very injurious to tobacco in Brazil and Central America [Moreira, 1923]. In Rhodesia, South Africa, etc. *Engytatus volucer* Kirk. is abundant and according to Roberts [1930] this species is the transmitter of the virus of leaf-curl of tobacco in Rhodesia. Roberts' results were however, not confirmed by Storey [1931] and Hopkins [1932] who worked in the same area.

18 636

Experiments with *Engytatus tenuis* during the 1934-35 season at Pusa were for want of proper apparatus, etc. restricted to *A* and *B* types of leaf-curl only (for description of the types see Dr. Pal's paper referred to above). It being essential that the diseased plants on which the insects are fed before they are transferred to healthy plants must definitely be of the specific types, Dr. Pal kindly supplied us with such plants at our request. He also raised for us seedlings under insect-proof covers which were subsequently transplanted in pots and covered with fine muslin bags in our laboratory and constituted the healthy stock for our transmission experiments.

---

*Recently the Imperial Institute of Entomology, London, has named this species as *Cyrtopeltus* (*Gallolicus*) *crassicornis* Dist. stated by China to be a synonym of Cyrtopeltis tenuis, Jan. '38

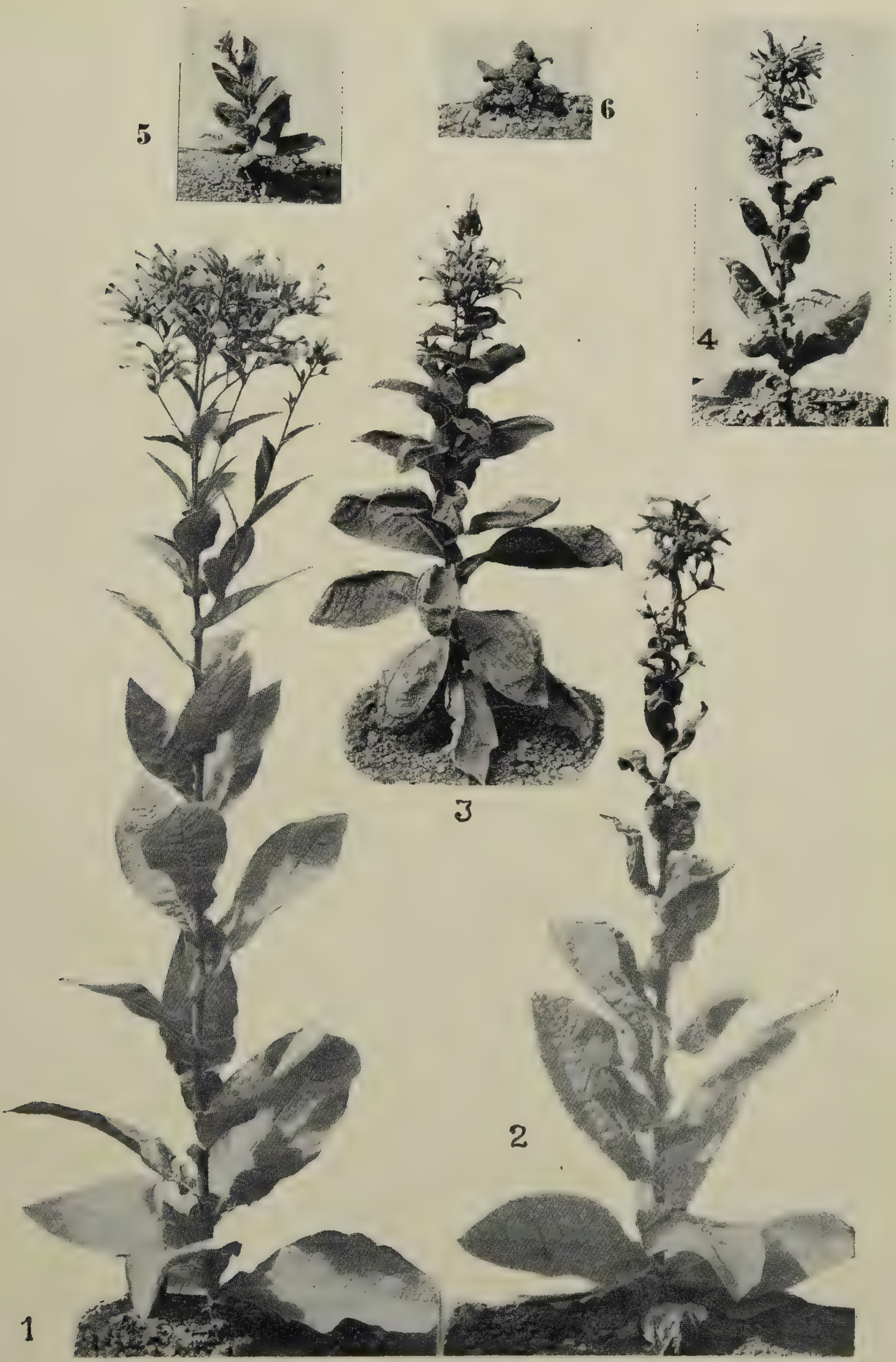


Fig. 1. A healthy tobacco plant (photo).

Figs. 2-6. Diseased tobacco plants (photo). The healthy and diseased plants were photographed with the camera being at the uniform distance. Note the tremendous decrease in size of the diseased plants.

The technique adopted in the experiments was as follows :—

The bugs of different stages of development were fed on diseased leaves enclosed in cellophane tubes for varying periods of time. They were then transferred along with the tubes to the leaves of healthy seedlings and allowed to live there till they died. When it was not possible to enclose the latter in cellophane tubes, fine meshed (thirty-five to an inch) wire-gauze cages of the size just enough to cover the seedling were used. These measures were adopted to ensure the feeding of the bugs on the enclosed seedlings. After a few days when the seedlings grew somewhat bigger, the wire-gauze cages were replaced by muslin bags. As the seedlings gradually grew still bigger, larger bags were substituted. Both healthy and diseased plants used in the experiments were of variety H 142.

Between 27th November and 25th December 1934 twenty-five transmission experiments for *A* type and fifteen for *B* type were performed with *Engytatus tenuis*. The healthy seedlings for these experiments had been transplanted in pots on 20th November, 1934. The details of this series of experiments are given in Appendix I. The bugs of both nymphal and adult stage were used and were fed on the diseased plants from three to nine days before they were transferred to healthy plants on which they lived for at least two days but some lived for over a month and in a few cases bred on the plants. The number of bugs fed on diseased plant and put on each healthy plant varied from two to ten. Twenty healthy plants were covered with muslin sleeves to serve as controls.

Again between the last week of December and the first week of January twenty experiments for *A* type and fifteen for *B* type were performed on the same lines as those stated in the above paragraph with fresh seedlings which had been transplanted on 12th December. Particulars of this series of experiments are given in Appendix II.

The inoculated and control plants of both series of experiments described above were kept under observation up to the middle of April 1935, when all of them were in flowers and some had even formed pods. Some plants showed an atypical growth, but none exhibited the symptoms of the leaf-curl disease.

Bugs used for the experiments performed during 1934-35 had been collected from the field as no pure stock of those bred in the laboratory was available when the work on leaf-curl was started in the autumn of 1934. During the summer of 1935 a stock of *Engytatus tenuis* was bred in the laboratory. During the following December and January fifty transmission experiments with this bug for *A*, *B*, *C* and *X* types of leaf-curl were performed, particulars of which are given in Appendix III. Both nymphal and adult stages of the bug were employed. For each experiment five specimens were allowed to feed on diseased plants for a period varying from one day to four days after which they were transferred to healthy seedlings on which they lived till their death, some having laid eggs and even given rise to nymphs in the meantime. The inoculated and the control plants were carefully examined up to the middle of April by which date all of them had reached the flowering stage. All the experiments gave negative results, no plant having developed symptoms of any type of the disease.

Roberts working in Rhodesia [1930] showed that if the juice of the thoracic region of *E. volucer* which had fed on diseased plants was injected into healthy tobacco seedlings, they developed symptoms of the leaf-curl disease. At Pusa we injected between 20th and 25th December 1935 about twenty young seedlings of tobacco with the juice of the thoracic region of *E. tenuis* which had previously been fed on diseased plants. The plants were kept under observation up to the middle of April 1936, but not a single one developed the disease.

Thus all the transmission experiments performed with *Engytatus tenuis* under the conditions described above gave negative results for the types of the disease examined. Thung [1932] also failed to get positive results with this species in Java. When the properties of the virus concerned in the leaf-curl disease are known, it will perhaps be possible to show conclusively if *Engytatus tenuis* is concerned in the transmission of the virus at all.

II. SANN-HEMP AS AN ALTERNATIVE HOST OF THE LEAF-CURL VIRUS AND THE INSECT CONCERNED WITH ITS TRANSMISSION TO TOBACCO

Sann-hemp is also a victim of virus diseases at Pusa. Its insect fauna has been under our observation since the end of 1934. During August 1935 this crop in the plots of the Imperial Economic Botanist, Pusa, was observed to be suffering from a virulent type of leaf-curl, resembling somewhat that of tobacco. The sucking insect which had been most common on this crop during the summer was the white-fly, *Bemisia gossypiperda*. To ascertain whether the virus responsible for the leaf-curl in sann-hemp is the same as that causing the disease in tobacco and whether the white-fly is the vector concerned, a series of transmission experiments were performed in the first half of September 1935. The technique and procedure were almost the same as those followed in the trial of *Engytatus tenuis* described above. Specimens of the white-fly (adults only) collected from sann-hemp plants were enclosed in cellophane tubes on the diseased leaves of the same plant for one to three days to make sure that they had sucked diseased juice. They were then transferred to young healthy tobacco seedlings (variety H 142) which had been grown under insect-proof muslin cages. To ensure their feeding on the healthy seedlings, they along with a leaf of the latter were enclosed in a cellophane tube which was removed after a couple of days, but the bugs were allowed to remain on the leaf till they died. The whole seedling remained under a muslin cover throughout. A number of seedlings from the same stock were kept under muslin bags to serve as controls. Detailed particulars of the various experiments are tabulated below :—

TABLE I

Particulars of transmission experiments with white-fly, Bemisia gossypiperda *M. & L.*

Variety of tobacco under experiment, H 142 ; date of germination, 17th June 1935 ; date of transplantation, 25th August 1935

1 Serial No. of experiment	2 Date of putting white flies on the diseased sann-hemp plant	3 Period for which the bugs sucked on the diseased plant (days)	4 No. of specimens transferred on healthy plants and their stage of development	5 Date of first appearance of some symptoms of the disease	6 Date when the disease was seen clearly on the inoculated plant	7 Remarks
1	30th Aug. 1935.	1	6 adults .	5th Nov. 1935	1st Dec. 1935	Disease virulent
2	30th Aug. 1935.	1	5 ,,	25th Sept. 1935.	5th Nov. 1935.	Disease virulent
3	31st Aug. 1935.	1	5 ,, .	...	...	No disease
4	31st Aug. 1935.	2	5 ,, .	25th Sept. 1935	...	The disease disappeared as the plant grew.
5	31st Aug. 1935.	2	4 ,, .	25th Sept. 1935.	...	Ditto
6	1st Sept. 1935	1	4 ,, .	...	...	No disease
7	1st Sept. 1935	1	4 ,, .	25th Sept. 1935	...	The disease disappeared as the plants grew.
8	1st Sept. 1935	1	5 ,, .	25th Sept. 1935.	5th Nov. 1935	Disease virulent
9	1st Sept. 1935	1	3 ,, .	...	...	No disease
10	3rd Sept. 1935	1	3 ,, .	...	...	No disease
11	4th Sept. 1935	1	4 ,, .	...	...	No disease
12	8th Sept. 1935	1	4 ,, .	...	...	No disease
13	7th Sept. 1935	2	4 ,, .	...	...	No disease
14	6th Sept. 1935	3	4 ,, .	...	...	No disease
15	9th Sept. 1935	1	5 ,, .	25th Sept. 1935	5th Nov. 1935	Disease virulent
16	9th Sept. 1935	1	5 ,, .	25th Sept. 1935.	5th Nov. 1935	Disease virulent
17	10th Sept. 1935.	1	6 ,, .	...	...	No disease
18	13th Sept. 1935.	1	6 ,, .	25th Sept. 1935.	5th Nov. 1935	Disease virulent
19	13th Sept. 1935.	1	4 ,, .	...	...	No disease
20	13th Sept. 1935.	1	6 ,, .	8th Oct. 1935	1st Dec. 1935	Disease virulent

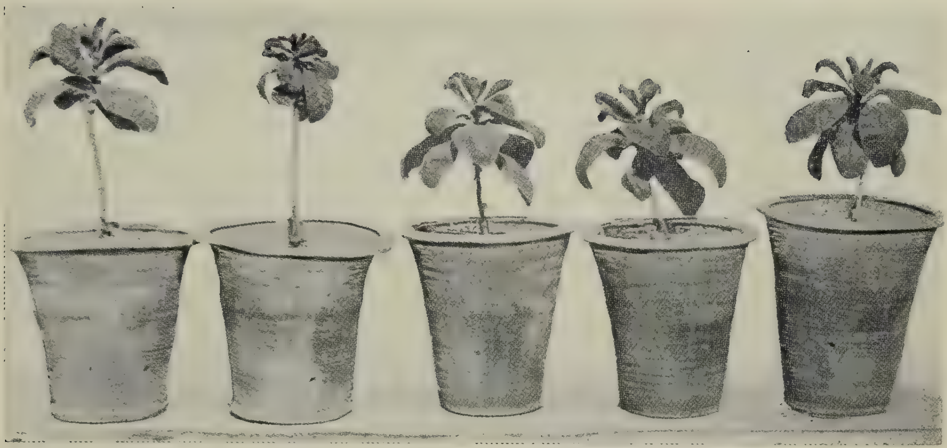
Controls 20 seedlings.

During the third or fourth week after the white flies had been put on the tobacco seedlings, ten out of twenty seedlings showed some crumpling of their top leaves and slight greening and thickening of the smaller veins thereof (See column 5 of the table). After four or five weeks three of these seedlings showed signs of recovery, whereas in the remaining seven the disease became more acute and developed all the important symptoms of *A* type leaf-curl. At no stage the disease could be designated as of any other type common at Pusa. Plate LVIII, figs. 1-5 illustrate the appearance of the diseased seedlings towards the end of December. The leaves of affected plants had got curled, twisted and puckered and the veins on their ventral sides had grown thick and developed distinct enations between them. Towards the end of March the seven diseased plants were only about one foot high, had hardly given out any flowers and had prominent leafy outgrowths of veins on the ventral sides. None of the control seedlings developed leaf-curl up to the end of the season but one of them from the very beginning showed symptoms of mosaic, which probably had got transmitted due to careless handling at the time of transplanting.

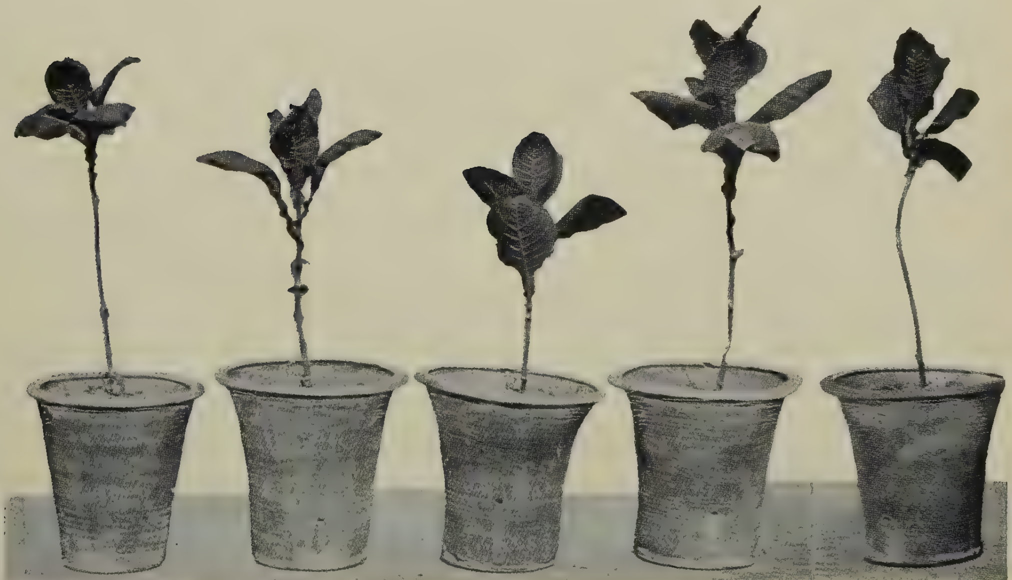
The above observations are of great interest as they clearly show that sann-hemp is one of the alternate hosts of the virus of the *A* type leaf-curl disease of tobacco and that the white-fly, *Bemisia gossypiperda*, is the vector concerned with its transmission to tobacco. It is likely that there are some other vectors also. It has yet to be ascertained whether the white-fly can transmit back the disease from tobacco to sann-hemp or from diseased tobacco to healthy tobacco. Furthermore, we have yet to investigate the role of the white-fly in the transmission of the virus or viruses of other types of leaf-curl common at Pusa.

Tobacco suffers from leaf-curl diseases in several parts of the world, having different names in various countries. In Rhodesia, Tanganyka, South Africa, etc. they are known as 'leaf-curl', 'leaf-crinkle', ' leaf-frenching ', etc. [Hopkins, 1932], while in Java and Sumatra they are designated as ' kroepoek ', etc. [Jochems, 1926 ; Thung, 1932 ; etc.].

It is not possible to say whether the leaf-curl diseases under our investigations are identical with those occurring in Africa and East Indies but it is of great interest to recall that in the latter countries also a white-fly, *Bemisia* sp., probably *B. gossypiperda*, is known to transmit the diseases [Thung, 1932-1934; Storey, 1931, 1932, etc.]



Figs. 1-5. Inoculated tobacco plants which developed leaf-curl by experiment, photographed three months after inoculation.



Figs. 6-10. Control plants of the same age and kept near the inoculated plants.

ACKNOWLEDGMENTS

We are very thankful to Dr. B. P. Pal, Second Economic Botanist, who is co-operating with us by studying the botanical aspects of the leaf-curl disease and has supplied us with various types of diseased plants and healthy seedlings for our transmission experiments. To the Assistant Agricultural Bacteriologist we are indebted for steam-sterilizing several samples of soil used for potting tobacco seedlings in our laboratory.

REFERENCES

70 Carter, W. (1927). *Jour. Agric. Res.* **34**.

349 Clayton, E. E. (1930). *Jour. Agric. Res.* **40**, 3.

Esaki, T. (1926). *Ann. Hist. Nat. Mus. Hung.* (*Budapest*) **24**, 136-189. [Abst. in *Rev. Appl. Ent.* (*Ser. A*) **15**, 506].

× Colding, F. D. (1930). *Empire Cotton Growing Review* **7**.

× Hopkins, J. C. F. (1932). *Mins. Min. Afric., S. Rhodesia, Bull.* No. **861**.

Jochems, S. C. J. (1926). *Med. Del. Proefstation, Medan-Sumatra, 2nd Ser.*, No. **43**.

Kunkel, L. O. (1926). *Amer. Jour. Bot.* **13**, 646-705.

Moreira, C. (1923). *Rep. Internat. Conf. Phytopath. & Econ. Ent.* (*Holland*), pp. 283-286. [Abst. in *Rev. Appl. Ent.* (*Ser. A*) **12**, 65].

Roberts, J. I. (1930). *Bull. Ent. Res.* **22**, 2. ? [illegible] [illegible]

Severin, H. H. P. (1934). *Hilgardia* **8**, No. 10, pp. 305-325, 329-335, 359-361. [Abst. in *Rev. Appl. Ent.* (*Ser. A*) **23**, 411-412].

Smith, K. M. (1933). 'Recent Advances in the Study of Plant Viruses': London.

Storey, H. H. (1932, 1) *Nature* **128**, 3222.

330 ———— (1932, 2). *Rhod. Agric. Jour.* **29**, 186-192.

717 ———— (1932, 3). *Proc. Roy. Soc.* (*B*) **112**, No. 774, p. 46.

381 Thung, T. H. (1932). *Proefstat. Voor Vorstenland. Tabak, Meded* (*Java*) No. **72**.

379 ————(1933). *Proefstat. Voor Vorstenland. Tabak, Meded* (*Java*), No. **78**.

Appendix I

Particulars of transmission experiments with Engytatus tenuis *Reut.* *with early-sown seedlings of tobacco during 1934-35*

Variety under experiment—H 142

Date of transplantation—27th November 1934

Serial No. of experiment	Date of putting bugs on the diseased tobacco	Type of disease	Period (in days) for which the bugs sucked on diseased tobacco	No. of specimens transferred to healthy tobacco and their stage of development	Results
1	27th Nov. 1934	*A*	9	2 adults . .	No disease appeared
2	28th Nov. 1934	*A*	8	5 ,, . .	,, ,, ,,
3	29th Nov. 1934	*A*	7	5 ,, . .	,, ,, ,,
4	30th Nov. 1934	*A*	7	5 ,, . .	,, ,, ,,
5	1st Dec. 1934 .	*A*	8	5 ,, . .	,, ,, ,,
6	7th Dec. 1934 .	*A*	7	5 ,, . .	,, ,, ,,
7	10th Dec. 1934	*A*	5	4 ,, . .	,, ,, ,,
8	13th Dec. 1934	*A*	4	1 nymph .	,, ,, ,,
9	15th Dec. 1934.	*A*	3	10 adults . .	,, ,, ,,
10	15th Dec. 1934.	*A*	3	1 ,, . .	,, ,, ,,
11	17th Dec. 1934.	*A*	3	1 nymph .	,, ,, ,,
12	17th Dec. 1934.	*A*	3	1 ,, . .	,, ,, ,,
13	18th Dec. 1934.	*A*	3	3 adults .	,, ,, ,,
14	19th Dec. 1934.	*A*	3	5 nymphs .	,, ,, ,,
15	19th Dec. 1934.	*A*	3	3 adults . .	,, ,, ,,
16	19th Dec. 1934.	*A*	3	3 nymphs .	,, ,, ,,
17	20th Dec. 1934.	*A*	3	5 adults . .	,, ,, ,,
18	20th Dec. 1934.	*A*	4	9 nymphs .	,, ,, ,,
19	22nd Dec. 1934	*A*	3	11 ,, . .	,, ,, ,,

APPENDIX I—*contd.*

Serial No. of ex-periment	Date of putting bugs on the diseased tobacco	Type of dis-sease	Period (in days) for which the bugs sucked on diseased tobacco	No. of specimens transferred to healthy tobacco and their stage of development	Results
20	22nd Dec. 1934	*A*	3	3 adults . .	No disease appeared
21	22nd Dec. 1934	*A*	3	2 ,, . .	,, ,, ,,
22	23rd Dec. 1934	*A*	3	5 nymphs .	,, ,, ,,
23	23rd Dec. 1934	*A*	5	5 ,, . .	,, ,, ,,
24	23rd Dec. 1934	*A*	5	1 ,, . .	,, ,, ,,
25	25th Dec. 1934.	*A*	3	2 adults and 2 nymphs.	,, ,, ,,
26	20th Dec. 1934.	*B*	2	7 nymphs .	,, ,, ,,
27	24th Dec. 1934.	*B*	6	7 nymphs and 1 adult.	,, ,, ,,
28	24th Dec. 1934.	*B*	6	6 nymphs and 1 adult.	,, ,, ,,
29	26th Dec. 1934.	*B*	5	8 nymphs and 1 adult.	,, ,, ,,
30	27th Dec. 1934.	*B*	4	10 nymphs only .	,, ,, ,,
31	27th Dec. 1934	*B*	4	8 nymphs and 1 adult.	,, ,, ,,
32	27th Dec. 1934.	*B*	4	5 nymphs and 3 adults.	,, ,, ,,
33	27th Dec. 1934.	*B*	4	5 adults . .	,, ,, ,,
34	28th Dec. 1934.	*B*	3	5 ,, . .	,, ,, ,,
35	28th Dec. 1934.	*B*	3	3 ,, . .	,, ,, ,,
36	31st Dec. 1934 .	*B*	3	9 ,, . .	,, ,, ,,
37	30th Dec. 1934.	*B*	4	4 ,, . .	,, ,, ,,
38	30th Dec. 1934.	*B*	4	5 ,, . .	,, ,, ,,
39	30th Dec. 1934.	*B*	4	3 ,, . .	,, ,, ,,
40	1st Jan. 1935 .	*B*	3	5 nymphs .	,, ,, ,,

APPENDIX II

Particulars of transmission experiments with Engytatus tenuis *Reut. with later-sown seedlings of tobacco during 1934-35*

Variety under experiment—H 142

Date of transplantation—12th December 1934

Serial No. of experiment	Date of putting bugs on the diseased tobacco	Type of disease	Period (in days) for which the bugs sucked on diseased tobacco	No. of specimens transferred to healthy tobacco and their stage of development	Results
1	25th Dec. 1934.	*A*	5	3 adults	No disease appeared
2	26th Dec. 1934.	*A*	4	2 ,,	,, ,, ,,
3	26th Dec. 1934.	*A*	4	1 ,,	,, ,, ,,
4	24th Dec. 1934.	*A*	7	2 nymphs	,, ,, ,,
5	25th Dec. 1934.	*A*	6	2 ,,	,, ,, ,,
6	29th Dec. 1934.	*A*	4	14 adults	,, ,, ,,
7	29th Dec. 1934.	*A*	4	7 ,,	,, ,, ,,
8	29th Dec. 1934.	*A*	4	2 nymphs and 3 adults.	,, ,, ,,
9	29th Dec. 1934.	*A*	4	5 adults	,, ,, ,,
10	29th Dec. 1934.	*A*	4	6 nymphs	,, ,, ,,
11	5th Jan. 1935	*A*	4	10 adults	,, ,, ,,
12	5th Jan. 1935	*A*	4	7 ,,	,, ,, ,,
13	5th Jan. 1935	*A*	4	6 ,,	,, ,, ,,
14	5th Jan. 1935	*A*	4	7 ,,	,, ,, ,,
15	5th Jan. 1935	*A*	4	9 ,,	,, ,, ,,
16	4th Jan. 1935	*A*	6	1 nymph	,, ,, ,,
17	4th Jan. 1935	*A*	6	1 nymph, became adult.	,, ,, ,,
18	16th Jan. 1935.	*A*	3	5 adults	,, ,, ,,
19	16th Jan. 1935.	*A*	3	11 ,,	,, ,, ,,
20	1st Jan. 1935	*B*	3	3 ,,	,, ,, ,,
21	2nd Jan. 1935	*B*	3	5 ,,	,, ,, ,,
22	2nd Jan. 1935	*B*	3	3 ,,	,, ,, ,,
23	2nd Jan. 1935	*B*	3	6 nymphs	,, ,, ,,
24	2nd Jan. 1935	*B*	3	8 adults	,, ,, ,,
25	3rd Jan. 1935	*B*	4	9 nymphs	,, ,, ,,
26	3rd Jan. 1935	*B*	4	5 ,,	,, ,, ,,
27	3rd Jan. 1935	*B*	4	5 adults	,, ,, ,,
28	3rd Jan. 1935	*B*	4	5 ,,	,, ,, ,,
29	3rd Jan. 1935	*B*	4	5 ,,	,, ,, ,,
30	3rd Jan. 1935	*B*	4	7 ,,	,, ,, ,,
31	4th Jan. 1935	*B*	4	5 ,,	,, ,, ,,
32	4th Jan. 1935	*B*	4	2 ,,	,, ,, ,,
33	4th Jan. 1935	*B*	4	3 ,,	,, ,, ,,
34	4th Jan. 1935	*B*	4	6 ,,	,, ,, ,,

APPENDIX III

Particulars of transmission experiments with Engytatus tenuis *Reut. during 1935-36*

Variety under experiment—H 142

Date of transplantation—14th October 1935

Serial No. of experiment	Date of putting bugs on diseased tobacco	Type of disease	Period (in days) for which the bugs sucked on diseased tobacco	No. of specimens transferred to healthy tobacco and their stage of development	Minimum period for which the bugs lived on healthy plants	Results
1	2nd Dec. 1935 .	*A*	4	5 nymphs, 5th instar	1 week .	No disease appeared
2	3rd Dec. 1935 .	*A*	3	5 " 3rd instar	1 " .	Do.
3	5th Dec. 1935 .	*A*	1	5 " 5th instar	1 " .	Do.
4	7th Dec. 1935 .	*A*	2	5 " 3rd instar	1 " .	Do.
5	7th Dec. 1935 .	*A*	2	5 adults . .	1 " .	Do.
6	7th Dec. 1935 .	*A*	2	5 nymphs, 3rd instar	1 " .	Do.
7	22nd Jan. 1936	*A*	3	5 adults . .	3 days .	Do.
8	22nd Jan. 1936 .	*A*	3	5 nymphs, 4th instar	3 " .	Do.
9	22nd Jan. 1936 .	*A*	5	5 " 4th instar	4 " .	Do.
10	22nd Jan. 1936 .	*A*	5	4 adults . .	4 " .	Do.
11	22nd Jan. 1936 .	*A*	5	5 nymphs, 3rd instar	4 " .	Do.
12	22nd Jan. 1936	*A*	5	5 adults . .	4 " .	Do.
13	2nd Dec. 1935 .	*B*	4	5 nymphs, 5th instar	1 week .	Do.
14	3rd Dec. 1935 .	*B*	3	5 " 3rd instar	1 " .	Do.
15	5th Dec. 1935 .	*B*	1	5 " 3rd instar	1 " .	Do.
16	6th Dec. 1935 .	*B*	3	5 adults . .	1 " .	Do.
17	7th Dec. 1935 .	*B*	2	5 " . .	1 " .	Do.
18	15th Dec. 1935	*B*	2	5 nymphs, 4th instar	1 " .	Do.
19	30th Nov. 1935	*C*	2	5 " 5th instar	1 " .	Do.
20	2nd Dec. 1935 .	*C*	4	5 " 5th instar	1 " .	Do.
21	21st Jan. 1936 .	*C*	4	5 " 4th instar	3 days .	Do.
22	21st Jan. 1936	*C*	4	5 adults . .	3 " .	Do.

APPENDIX III—*contd.*

Serial No. of experiment	Date of putting bugs on diseased tobacco	Type of disease	Period (in days) for which the bugs sucked on diseased tobacco	No. of specimens transferred to healthy tobacco and their stage of development	Minimum period for which the bugs lived on healthy plants	Results
23	22nd Jan. 1936	*C*	3	5 nymphs, 4th instar	3 days .	No disease appeared
24	22nd Jan. 1936	*C*	3	5 adults . .	3 ,, .	Do.
25	30th Nov. 1935	*D*	2	5 nymphs, 5th instar	1 week .	Do.
26	2nd Dec. 1935 .	*D*	4	5 ,, . 5th instar	1 ,, .	Do.
27	3rd Dec. 1935 .	*D*	3	5 ,, 3rd instar	1 ,, .	Do.
28	5th Dec. 1935 .	*D*	1	5 nymphs, 5th instar, became adults.	1 ,, .	Do.
29	5th Dec. 1935 .	*D*	1	5 nymphs, 3rd instar.	1 ,, .	Do.
30	5th Dec. 1935 .	*D*	1	5 ,, 5th instar, became adults.	1 ,, .	Do.
31	6th Dec. 1935 .	*D*	3	5 adults . .	1 ,, .	Do.
32	7th Dec. 1935 .	*D*	2	5 ,, . .	1 ,, .	Do.
33	7th Dec. 1935 .	*D*	2	5 ,, . .	1 ,, .	Do.
34	14th Dec. 1935	*D*	2	5 ,, . .	1 ,, .	Do.
35	14th Dec. 1935.	*D*	2	5 nymphs, 4th instar	1 ,, .	Do.
36	14th Dec. 1935	*D*	2	5 adults . .	1 ,, .	Do.
37	14th Dec. 1935	*D*	2	5 ,, . .	1 ,, .	Do.
38	15th Dec. 1935	*D*	2	5 nymphs, 4th instar	1 ,, .	Do.
39	15th Dec. 1935	*D*	2	5 ,, 4th instar	1 ,, .	Do.
40	15th Dec. 1935	*D*	2	5 adults . .	1 ,, .	Do.
41	18th Dec. 1935	*D*	1	5 nymphs, 4th instar	1 ,, .	Do.
42	18th Dec. 1935	*D*	1	5 ,, 4th instar	3 days .	Do.
43	21st Dec. 1935.	*D*	4	5 ,, 4th instar	3 ,,	Do.

ABSTRACT

Soil deterioration in the canal irrigated areas of the Punjab. Part I. Equilibrium between Ca and Na ions in base exchange reactions. Part II. Relation between degree of alkalisation and dispersion coefficient in deteriorated soil. E. McKenzie Taylor, Amar Nath Puri and A. G. Asghar. (*Punjab Irrigation Research Institute Research Publications*, Vol. **4**, Nos. 7 and 8).

IRRIGATION AND SOIL DETERIORATION

In areas of low rainfall, an equilibrium between the soil, climate and crop is established as the result of a natural adjustment among the several agencies that have been operating over a long series of years. The introduction of irrigation into such areas results in the upsetting of this equilibrium and is inevitably followed sooner or later by rise in the subsoil water and soil deterioration from the point of view of crop production.

The introduction of canal irrigation in the Punjab is followed by a gradual rise in the level of the subsoil water causing soil deterioration by the accumulation of sodium salts in the surface soil. In some parts of the province in which underground drainage system has been introduced, it has become necessary to dispose of the water by pumping it back into the canals. It is, therefore, of importance to investigate the safe limits for the salt content of the irrigation water which can be used without risk of injury to the soil. It is also of importance to examine the possibilities of adopting measures for the prevention of soil deterioration, under irrigation.

Dr. McKenzie Taylor and Dr. Amar Nath Puri of the Punjab Irrigation Research Institute have been studying these problems and have discussed their results in two publications of the Institute, " Soil deterioration in the canal irrigated areas of the Punjab." (Punjab Irrigation Research Institute Publication, Vol. IV, Nos. 7 and 8). The first part deals with the equilibrium between calcium and sodium ions in exchange reactions in the soil. The effect of the amount of its exchangeable Na on the quality and agricultural value of a soil is expressed by what is called 'the Degree of Alkalisation (D. A.)', which is the ratio of the amount of exchangeable sodium to the maximum amount of sodium that a soil is capable of holding.

An examination of the yields of wheat and rice of several experimental plots showed that as the D. A. of the soil increases, the yield decreases proportionately. The yield was also found to be negatively correlated with the *p*H of the soil and beyond *p*H 8·5 it was much below par, indicating that soil deterioration had set in.

Treatment of a calcium-saturated soil with solutions of sodium chloride of increasing concentration brought out the interesting fact that, as the concentration of the sodium chloride increases, a relatively smaller amount of calcium is sufficient to prevent its interaction with the soil as regards replacement. The application of a saturated solution of calcium sulphate, the strength of which was found to be only $N/25$ could completely prevent alkalisation by a solution containing as much as 300 to 400 parts per 100,000 of sodium chloride. The application of this substance, therefore, at a stage when the soil begins to deteriorate (pH 8·5) will prevent further entry of Na in the exchange complex.

Irrigation waters, devoid of calcium and containing even only 60 parts per 100,000 of sodium chloride are harmful, but if calcium salts are present, the limit can be considerably exceeded without being detrimental to the soil.

The quality of the soil, especially with regard to its Na : Ca ratio, should be taken into consideration while considering the suitability or otherwise of water for irrigation.

The second part deals with the relation between the degree of alkalisation and the dispersion coefficient in deteriorated soils. This coefficient is measured by determining the percentage of the total clay which remains in suspension after twenty-four hours. The dispersion coefficient varied with different soils. In some soils the dispersion coefficient attained its maximum value when the degree of alkalisation was about 60 per cent. When, however, an acid treated soil was saturated with calcium and then subjected to treatment with varying concentrations of sodium hydroxide, the dispersion coefficient reached its maximum value when the degree of alkalisation was only 30 per cent. Under similar treatment, the behaviour of laterite soils was in marked contrast to the other soils examined. Even when the soil was fully saturated with sodium, the maximum limit of dispersion was not reached. [B. V. N.]

NOTE

FIRST GUIDE TO AGRICULTURAL LITERATURE

The following communication has been received from the Press Bureau of the International Institute of Agriculture, Villa Umberto, Rome :—

The Library of the International Institute of Agriculture in Rome, one of the leading centres of world literature in agriculture and allied subjects, has compiled and published a complete list* of current bibliographical sources, which are available for these subjects in all the various countries of the world. Considering the overwhelming output of printed matter in form of books, bulletins, reports, periodicals, etc., the importance of bibliographies is quite evident. They are the keys which unlock the immense wealth of literary production. The compilation of a complete list of existing bibliographical sources is therefore an event of considerable importance not only for the agricultural research worker in the strict sense but also for the scientists of the many fields which deal indirectly with agricultural matters.

The newly published list is the outcome of co-operative work of the staff of the Institute's Library and the International Committee of Agricultural Librarians recently founded. The final arrangement and the text of the notes, which give a detailed description of the contents, scope, inclusiveness, classification and other features of each bibliography, is the work of Mr. V. A. Schaefer, under the direction of Dr. S. V. Frauendorfer, Chief Librarian of the Institute.

The list contains not only pure title bibliographies, but also abstracting journals and bibliographical sections, contained in other periodicals or annuals. The titles are arranged by countries of publication. A separate section includes publications of International Institutions. An alphabetical subject index and a title index facilitate greatly the use of the list, which gives information on more than 200 titles of 26 different countries. The publication is bilingual, English and French combined.

---

* A Survey of Current Bibliographies on Agriculture and Allied Subjects. Rome, 1937. Price 10 Liras.

QUARANTINE ANNOUNCEMENT

The following notification (No. F.-116/34 A., dated the 8th April 1937) issued by the Government of India in the Department of Education, Health and Lands, is reproduced for general information :—

In continuation of this Department Notification No. 360, dated the 29th February 1924, as subsequently amended, it is notified for general information that no charge will be made for the consignments of plants intended for export to countries abroad, which are sent to the Government Fruit Farm, Mirpurkhas, for inspection and certification provided that all incidental charges are borne by the exporters. Consignments may also be made available for inspection and certification work at Karachi. The Chief Agricultural Officer in Sind is authorised to charge a fee of Rs. 20 for each such consignment.

THE MAYNARD-GANGA RAM PRIZE

Applications are invited for the 'Maynard-Ganga Ram Prize' of the value of Rs. 3,000 which will be awarded for a discovery, or an invention, on a new practical method tending to increase agricultural production in the Punjab on a paying basis. The prize is open to all, irrespective of caste, creed or nationality and Government servants are also eligible for it. Essays and theses are not eligible for competition and applicants should prove that some part of their discovery, invention, etc., is the result of work done after the prize was founded in 1925. The Managing Committee reserves to itself the right of withholding or postponing the prize, if no satisfactory achievement is reported to it. All entries in competition for the next award should reach the Director of Agriculture, Punjab, Lahore, on or before the 31st December, 1938.

RECENT PUBLICATIONS OF THE IMPERIAL AGRICULTURAL BUREAUX

I. OBTAINABLE FROM THE IMPERIAL BUREAU OF SOIL SCIENCE, ROTHAMSTED EXPERIMENTAL STATION, HARPENDEN, HERTS

Periodical Abstracts

Lists of publications and papers on Soil Science published in the Empire Overseas in :—

	Price *s.*	*d.*
1933	1	0
1934	1	0
Soil Research in the British Empire published during 1935	1	0
Lists of Publications relating to Soils and Fertilisers—		
Published monthly, per annum, post free	10	0
Monthly Letters—		
Free to recipients, within the British Empire, of 'Publications relating to Soils and Fertilisers.' Subscription, outside the Empire, per annum	4	0
Recent Developments in Soil Analysis—		
Quarterly Supplement to the above publications. Separate copies each	0	6

Occasional Papers

	s.	*d.*
Technical Communications—		
34. Tropical Soils in relation to Tropical Crops	2	6
Annual Report : For the year 1934-35	0	6
Bibliographies—		
Bibliography on Coffee	2	0
Catalogue of Journals and Periodicals in the Library of Rothamsted Experimental Station	2	0
Special Publication—		
The Katamorphism of Igneous Rocks under Humid Tropical Conditions (by the late Sir J. B. Harrison)	5	0
Bibliography of Soil Science, Fertilizers and General Agronomy, 1931-34	25	0

II. OBTAINABLE FROM THE IMPERIAL BUREAU OF ANIMAL HEALTH, VETERINARY RESEARCH LABORATORY, NEW HAW, WEYBRIDGE, SURREY

Abstracting Journal

	s.	*d.*
The Veterinary Bulletin—		
1931. Vol. 1. Quarterly (1st Number, April)	7	6
Annual Subscription	20	0
Subsequent Volumes. Monthly (1st Number, January)	5	0
Annual Subscription (postage paid)	40	0

Indexing Publication

	Price s.	 d.
Index Veterinarius—Four issues a year. First issue April 1933—		
Annual Subscription (postage paid). Volumes I to III mimeographed, Volume IV onwards printed	100	0

III. OBTAINABLE FROM THE IMPERIAL BUREAU OF ANIMAL NUTRITION, ROWETT RESEARCH INSTITUTE, BUCKSBURN, ABERDEEN

Journal

Nutrition Abstracts and Reviews. (Issued under the direction of the Imperial Agricultural Bureaux Council, the Medical Research Council and the Reid Library)—		
Subscription per volume of 4 numbers	42	0
Per single number	13	0

Occasional Papers

Technical Communications—		
7. Wheat : Pre-eminence as a Cereal Food, Nutritive Value ; Relation to Health and Disease	1	0
Occasional Communications—		
1. The Effect of Climate on the Composition of Pasture Plants		

IV. OBTAINABLE FROM THE IMPERIAL BUREAU OF PLANT GENETICS (FOR CROPS OTHER THAN HERBAGE), PLANT BREEDING INSTITUTE, SCHOOL OF AGRICULTURE, CAMBRIDGE

Journal

Plant Breeding Abstracts—		
Quarterly. Annual Subscription	15	0
Single copies	5	0

Occasional Papers

Indexes to Plant Breeding Abstracts—		
Subject Index to Vols. I to V of Plant Breeding Abstracts	5	0
Supplements to Plant Breeding Abstracts—		
Summary of Reports received from Countries exclusive of the British Empire, 1928-31. Supplement I	2	6
Summary of Reports received from Stations in the British Empire, 1932-1935. Supplement II	5	0
Technical Communications—		
Vernalization and Phasic Development of Plants (Joint Publication of the Imperial Bureaux of Plant Genetics)	10	0
Bibliographical Monographs—		
The Experimental Production of Haploids and Polyploids	5	0
Breeding Resistant Varieties, 1930-33 (Supplement)	2	0
An Outline of Cytological Technique for Plant Breeders	1	6

V. Obtainable from the Imperial Bureau of Plant Genetics (Herbage Plants), Welsh Plant Breeding Station, Agricultural Buildings, Alexandra Road, Aberystwyth, Wales

Journals

	Price	
	s.	*d.*
Herbage Abstracts—		
Quarterly. Annual Subscription	15	0
Single numbers	5	0
Herbage Reviews—		
Subscription is at present—Vol. 1 (1933), Vol. 2 (1934), Vol. 3 (1935), Vol. 4 (1936)—included in that to Herbage Abstracts		

Occasional Papers

Bulletins—		
17. Vernalization and Phasic Development of Plants. December, 1935. (Joint publication of the Imperial Bureaux of Plant Genetics)	10	0

VI. Obtainable from the Imperial Bureau of Fruit Production, East Malling Research Station, East Malling, Kent

Journal

Horticultural Abstracts—		
A quarterly abstract publication of current horticultural literature—		
Annual Subscription	15	0
Single copy	4	0

Technical Communications

8. Horticultural Aspects of Woolly Aphis Control together with a Survey of the Literature. R. M. Greenslade	2	6

Occasional Papers

3. Annotated Bibliography on Bitter-Pit. 1934	1	6
Other Publications—		
Index to Volumes I-X of the *Journal of Pomology and Horticultural Science*, 1933. Compiled by Bureau, published by the Editors of the *Journal of Pomology and Horticultural Science*. Available from the Bureau	5	0
Old and New standpoints on senile degeneration, 1931. By A. P. C. Bijhouwer	0	6

VII. Obtainable from the Imperial Bureau of Animal Genetics, Institute of Animal Genetics, University of Edinburgh, King's Buildings, West Mains Road, Edinburgh

Journal

Animal Breeding Abstracts (Quarterly) Commencing April, 1933. Annual Subscription	15	0

Occasional Papers

	Price s.	Price d.
Animal Breeding in the British Empire. A Survey of Research and Experiment, 1934	2	0

VIII. OBTAINABLE FROM THE IMPERIAL BUREAU OF AGRICULTURAL PARASITOLOGY, INSTITUTE OF AGRICULTURAL PARASITOLOGY, WINCHES FARM DRIVE, HATFIELD ROAD, ST. ALBANS, HERTS

Journals

	s.	d.
Bibliography of Helminthology. For the year 1933—		
Library Edition, bound in cloth	10	6
Stiff paper cover only	8	0
Helminthological Abstracts. (Issued in five or six parts per volume, each volume covering the literature of a single year.) Subscription per volume, payable in advance	30	0
Completed volumes bound in cloth	32	0

Occasional Papers

	s.	d.
The Effects of some Natural Factors on the Second Ecdysis of Nematode Infective Larvae. By G. Lapage. 25 pp.	4	0
Notes and Memoranda—		
11. Recent Developments in the Control of *Heterodera marioni*. 6 pp	1	6

PUBLICATIONS ISSUED BY THE IMPERIAL INSTITUTE OF ENTOMOLOGY, 41, QUEEN'S GATE, LONDON, S. W. 7

Bulletin of Entomological Research.—Published quarterly and containing original articles on Economic Entomology. Issued post-free.

	s.	d.
Annual subscription (payable in advance)	30	0

Review of Applied Entomology.—Consisting of abstracts or reviews of all current literature on Economic Entomology throughout the world. Published monthly in two series. Series 'A' dealing with Insect and other Arthropod pests of cultivated plants, forest trees and stored products of animal and vegetable origin, Series 'B' dealing with insects, ticks, etc., conveying disease or otherwise injurious to man and animals. Issued post-free.

	Series 'A' s.	Series 'B' s.
Annual subscription (payable in advance)	30	15
Vol. XXIII (1935)	42	21

	Price *s.* *d.*
Zoological Record—Part Insecta.—Published annually about July in each year and containing as complete a record as possible of the literature of the previous year chiefly from the systematic standpoint.	
Annual subscription (including postage)	15 6
Report of the Fourth Imperial Entomological Conference, 19-27th September 1935	4 0

PUBLICATIONS OBTAINABLE FROM THE IMPERIAL MYCOLOGICAL INSTITUTE, KEW, SURREY

Journal

	Price *s.* *d.*
Review of Applied Mycology.—Annual subscription, 12 monthly parts, with title page and index (post-free)	21 0
Single part	2 0
Title page and index	3 0
Report on the Third Imperial Mycological Conference, 1934 . . .	2 0

PUBLICATIONS OF THE IMPERIAL COUNCIL OF AGRICULTURAL RESEARCH, INDIA

---

1. Agriculture and Live-stock in India

A bi-monthly journal of agriculture and animal husbandry for the general reader interested in agriculture or live-stock in India or the Tropics. (Established 1931. Published in January, March, May, July, September and November. Prepayable subscription Rs. 6 or 9*s*. 9*d*. per annum inclusive of Indian postage. Price per part Rs. 2 or 3*s*. 6*d*. inclusive of Indian postage.) Volumes I to VI complete are available.

2. The Indian Journal of Agricultural Science

A bi-monthly scientific journal of agriculture and the allied sciences, mainly devoted to the publication of the results of original research and field experiments. (Established 1931. Published in February, April, June, August, October and December. Prepayable subscription Rs. 15 or 24*s*. per annum inclusive of Indian postage. Price per part Rs. 3 or 5*s*. 3*d*. inclusive of Indian postage.) Volumes I to VI complete are available.

3. The Indian Journal of Veterinary Science and Animal Husbandry

A quarterly journal for the publication of scientific matter relating to the health, nutrition and breeding of live-stock. (Established 1931. Published in March, June, September and December. Prepayable subscription Rs. 6 or 9*s*. 9*d*. inclusive of Indian postage. Price per part Rs. 2 or 3*s*. 6*d*. inclusive of Indian postage.) Volumes I to VI complete are available.

4. Scientific Monographs of the Imperial Council of Agricultural Research

NCAG—SM/1	No. 1. The Fungi of India, By E. J. Butler, C.I.E., D.Sc., M.B. F.R.S. and G. R. Bisby, Ph.D. (1931). Price Rs. 6-12-0 or 11*s*. (As. 8.)
NCAG—SM/2	No. 2. Life-histories of Indian Microlepidoptera. Second Series: Alucitidae (Pterophoridae, Tortricina and Gelechiadae), By T. Bainbrigge Fletcher, R.N., F.L.S., F.E.S., F.Z.S. (1932). Price Rs. 3-4-0 or 5*s*. 6*d*. (As. 6.)
NCAG—SM/3	No. 3. The Open Pan System of White-Sugar Manufacture, By R. C. Srivastava, B.Sc. (1935. 2nd edition). Price Rs. 3-2-0 or 5*s*. 6*d*. (As. 9.)
NCAG—SM/4	No. 4. Life-histories of Indian Microlepidoptera : Cosmopterygidae to Neopseustidae. By T. Bainbrigge Fletcher, R.N., F.L.S., F.E.S., F.Z.S. (1933). Price Rs. 4-8-0 or 7*s*. 6*d*. (As. 8.)
NCAG—SM/5	No. 5. The Bombay Grasses. By E. J. Blatter, S.J., Ph.D. F.L.S. and C. McCann, F.L.S. Illustrated by R. K. Bhide. Price Rs. 20-12-0 or 32*s*. 6*d*.
NCAG—SM/6	No. 6. Helminth Parasites of the Domesticated Animals in India, By G. D. Bhalerao, M.Sc. Price Rs. 7-12-0 or 13*s*. 3*d*.
NCAG—SM/7	No. 7. Influence of Manures on the Wilt Disease of *Cajanus indicus* Spreng. and the isolation of Types Resistant to the Disease, By W. McRae, M.A., D.Sc.(Edin.), F.L.S. and F. J. F. Shaw, D.Sc. (Lond.), A.R.C.S., F.L.S. (1933). Price Rs. 2-4-0 or 4*s*. 3*d*. (As. 5.)

NCAG—SM/8	No. 8. The Silk Industry of Japan with Notes on Observations in the United States of America, England, France and Italy, By C. C. Ghose, B.A., F.E.S. (1933). Price Rs. 4 or 6*s.* 9*d.* (As. 7).
NCAG—SM/9	No. 9. Mechanical Cultivation in India. A History of the Large Scale Experiments carried out by Burmah-Shell Oil Storage and Distributing Company of India, Ltd., By C. P. G. Wade. Price Rs. 3-14-0 or 6*s.* 6*d.*
NCAR—SM/10	No. 10. Spotted Boll-worms of Cotton (*Earias fabia* Stoll and *Earias insulana* Boisd.) in South Gujarat, (Final report on investigations financed by the Indain Central Cotton Committee, 1923-1931), By B. P. Deshpande, M.Ag. and N. T. Nandkarny. Price Rs. 5-14 or 9*s.* 6*d.*
ICAR—10-11	No. 11. Investigations on the Course and Distribution of the Nerve supplying Levator anguli scapuli and Rhomboideus Muscles and the Formation of the Phrenic Nerve in the Ox, with observations on certain Anatomical Deviations, by H. N. Chelva Ayyangar, G. M. V. C. Price Rs. 4-10 or 7*s.* 9*d.*

5. Miscellaneous Bulletins of the Imperial Council of Agricultural Research

NCAG—MB/1	No. 1. List of Publications on Indian Entomology (1930), Compiled by the Imperial Entomologist, Pusa. Price As. 14 or 1*s.* 6*d.*
NCAG—MB/2	No. 2. List of Publications on Indian Entomology (1931), Compiled by the Imperial Entomologist, Pusa. Price As. 8 or 10*d.*
NCAG—MB/3	No. 3. List of Publications on Indian Entomology (1932), Compiled by the Imperial Entomologist, Pusa. Price As. 12 or 1*s.* 3*d.*
NCAG—MB/4	No. 4. Host Plant Index of Indo-Ceylonese Coccidae, By S. Ramachandran, L.Ag. and T. V. Ramakrishna Ayyar, B.A., Ph.D., F.Z.S. Price Re. 1-10 or 2*s.* 9*d.*
NCAG—MB/5	No. 5. List of Publications on Indian Entomology (1933), Compiled by the Officiating Imperial Entomologist, Pusa. Price As. 9 or 1*s.*
NCAG—MB/6	No. 6. Bee-keeping, By. C. C. Ghosh, B.A., F.E.S. (3rd Edition.) Price Re. 1-14 or 3*s.* 3*d.*
NCAG—MB/7	No. 7. List of Publications on Indian Entomology (1934), Compiled by the Officiating Imperial Entomologist and the Imperial Entomologist, Pusa. Price Re. 1-2-0 or 2*s.*
NCAR—MB/8	No. 8. Selected Clinical Articles, by G. K. Sharma, G.P.V.C and R. L. Kaura, B.V.Sc., M.R.C.V.S. Price As. 8 or 10*d.*
NCAR—MB/9	No. 9. Statistical Methods and their Application to Agronomy. A Bibliography, By K. K. Guha Roy, B. A. Price Rs. 2-2 or 4*s.*
NCAR—MB/10	No. 10. Diseases of Sugarcane and Methods for their Control, By L. S. Subramaniam. Price Re. 1-14 or 3*s.* 3*d.*
NCAR—MB/11	No. 11. Tables of Standard Errors of Mendelian Ratios, By Swarn Singh Purewal, M. Sc., Ph. D. and P. Krishna Rao, L. Ag. Price As. 12 or 1*s.* 3*d.*
NCAR—MB/12	No. 12. List of Publications on the Botany of Indian Crops, Part II for the period 1928-32, By R. D. Bose. Price Rs. 3-6-0 or 5*s.* 9d.
NCAR—MB/13	No. 13. Two New Statistical Tables based upon Fisher's '*t*', By M. Vaidyanathan. Price As. 6 or 8*d.*
NCAR—MB/14	No. 14. List of Publications on Indian Entomology, Compiled by the Imperial Entomologist. Price Re. 1-4-0 or 2*s.*
NCAR—MB/15	No. 15. Selected Clinical Articles, Part II. (*In the Press.*)
NCAR—MB/16	No. 16. Indian Grazing Conditions and the Mineral Contents of some Indian Fodders, By P. E. Lander, M.A., D.Sc., F. I. C., I. A. S. (*In the Press*).

6. Annual Reports of the Imperial Council of Agricultural Research

NCAG—AR/31	Annual Report of the Imperial Council of Agricultural Research for the years 1929-30 and 1930-31. Price As. 12 or 1*s.* 3*d.* (Anna 1.)
NCAG—AR/32	Annual Report of the Imperial Council of Agricultural Research for the year 1931-32. Price As. 6 or 8*d.* (As. 2.)
NCAG—AR/33	Annual Report of the Imperial Council of Agricultural Research for the year 1932-33. Price As. 6 or 8*d.*
NCAG—AR/34	Annual Report of the Imperial Council of Agricultural Research for the year 1933-34. Price Re. 1-8 or 2*s.* 6*d.*
NCAG—AR/35	Annual Report of the Imperial Council of Agricultural Research for the year 1934-35. Price Re. 1 or 1*s.* 9*d.*
NCAR—AR/36	Annual Report of the Imperial Council of Agricultural Research for the year 1935-36. Price As. 14 or 1*s.* 6*d.*
ICAR—12-37.	Annual Report of the Imperial Council of Agricultural Research for the year 1936-37. Price Re. 1 as. 2 or 2*s.*

7. Agriculture and Animal Husbandry in India (called Review of Agricultural Operations in India up to 1933)

NCAG—R. 1/29	Review of Agricultural Operations in India, 1928-29. Price Rs. 3-2 or 5*s.* 6*d.* (As. 8.).
NCAG—R. 1/31	Review of Agricultural Operations in India, 1929-31. Price Rs. 5 or 8*s.* 3*d.* (As. 10.)
NCAG—R. 1/33	Review of Agricultural Operations in India, 1931-33. Price Rs. 5-12 or 9*s.* 6*d.*
NCAG—R. 1/35	Agriculture and Animal Husbandry in India, 1933-35 : Part I—Crop Production. Price Rs. 4-14-0 or 8*s.* Part II—Animal Husbandry. Price Re. 1 or 1*s.* 9*d.*

8. Proceedings of the Board of Agriculture and Animal Husbandry

NCAG—P. 1	Proceedings of the First Meeting of the Animal Husbandry Wing of the Board of Agriculture and Animal Husbandry held at New Delhi from 20th to 23rd February 1933, with Appendices. Price Rs. 5-14-0 or 9*s.* 6*d.*
NCAR—P. 2	Proceedings of the First Meeting of the Crops and Soils Wing of the Board of Agriculture and Animal Husbandry in India held at Delhi from 25th February to 2nd March 1935, with Appendices. Price Rs. 6 or 9*s.* 9*d.*

A Description of the Imperial Institute of Veterinary Research, Muktesar and its sub-station, the Imperial Veterinary Serum Institute, Izatnagar, By F. Ware, F.R.C.V.S. Price Re. 1-4 or 2*s.*

The Production of Cigarette Tobacco by Flue-curing, By F. J. F. Shaw, C.I.E., D.Sc., A.R.C.S., F.L.S. and Kashi Ram, *Imp. Inst. Agri. Res. Pusa Bull.* No. 187. Reprinted (1935). Price Re. 1 or 1*s.* 9*d.*

A Handbook of Statistics for use in Plant Breeding and Agricultural Problems, By F. J. F. Shaw, C.I.E., D.Sc., A.R.C.S., F.L.S. Price Rs. 4-6-0 or 7*s.* 3*d.*

[Copies of the above publications can be had from :—

The Manager of Publications, Civil Lines, Delhi, India.

In Europe and America—From Public Department (Publications Branch), Office of the High Commissioner for India, India House, Aldwych, London, W. C. 2.

GIPD—S3—52 IC of AR—10 8-37—800.

Nd

ORIGINAL ARTICLES

CYTOLOGICAL STUDIES IN PADDY VARIETIES

BY

B. L. SETHI, M.Sc., Ph.D. (Wales)

Assistant Paddy Specialist, Rice Research Station, Nagina

(Received for publication on 30th March 1936)

(With Plates LIX-LXI)

I. Introduction

Up to date there has not been much work done in India on rice cytology. A good deal of work, however, has been done on Japanese varieties by workers in Japan. Kuwada [1910] in his studies on *Oryza sativa* L. reported twelve as its reduced number of chromosomes. Nakatomi [1923] made a more detailed study, comprising a wide range of forms, possessing diverse characteristics and found them all consistent in chromosome numbers. Kato [1930] in his investigations of seven *japonica* and *indica* types established twelve as the haploid number of chromosomes in every case. Selim [1930] examined five different forms of the species and found them all identical in number. Later, Morinaga and Fukishima [1931] in their more extensive study of twenty-five varieties, observed no difference in their chromosome counts. There seems considerable scope for exploring the cytology of Indian rices ; only two or three strains have so far been examined and even in these a detailed study has not been made. Rau [1929] examined two strains of *Oryza sativa* and found twenty-four chromosomes in the root tip. He was, however, unable to obtain haploid counts despite repeated endeavours. Ramiah [1932], in a very short description, reported twelve as the haploid number of chromosomes.

A particular species of paddy is usually represented by several forms or varieties. The primary object of such investigations, in this wide range of forms, is to ascertain whether the extreme forms differ in chromosome number, and if not, what differences in the behaviour of chromosomes during meiosis or what morphological variations in the chromosomes themselves are connected with the observed varietal differences. Further, the cytological work here presented was undertaken with a view to obtain data that might have a bearing on the phylogeny of the various types as well as on their taxonomic relationship with each other.

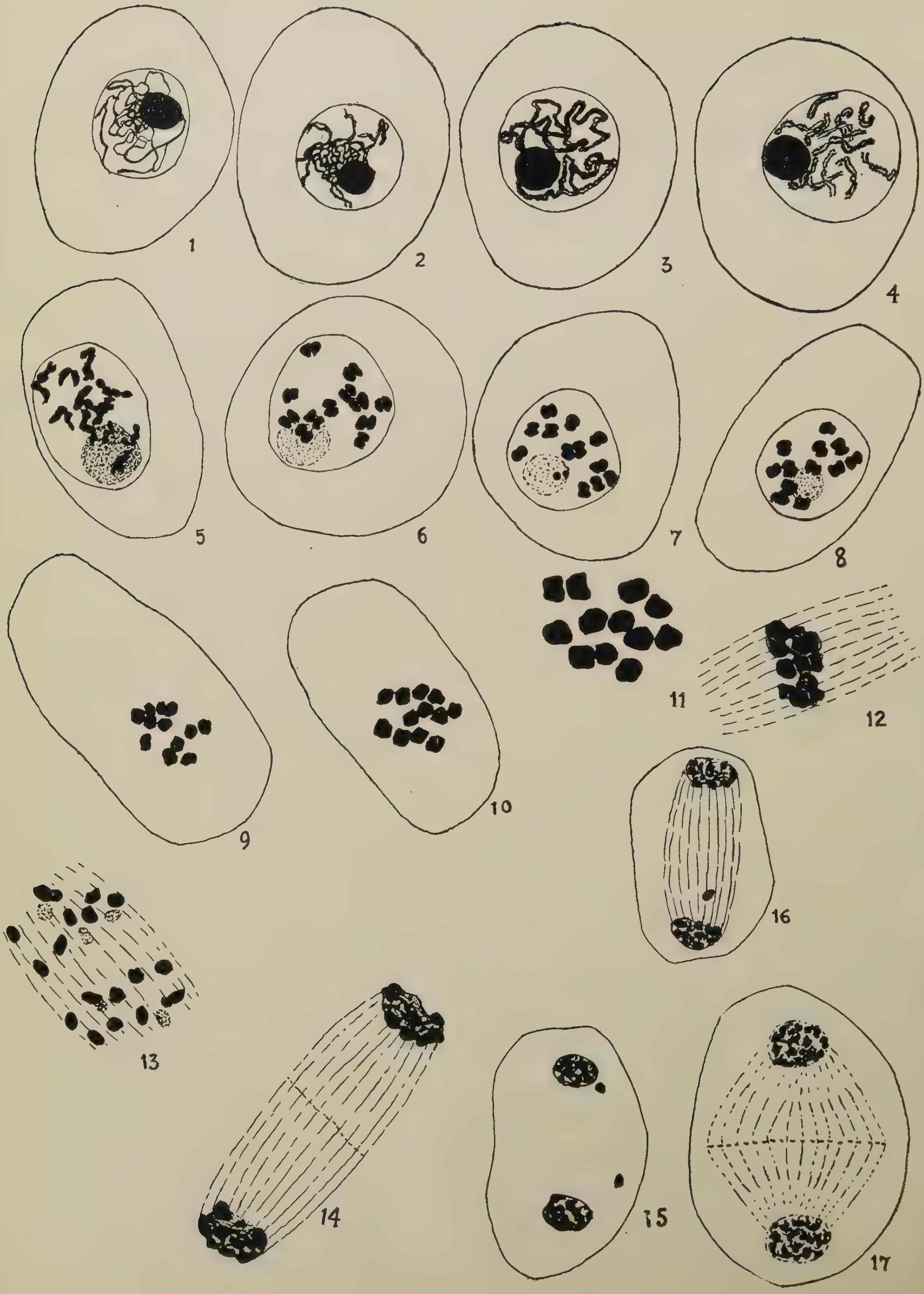
II. MATERIAL AND METHODS

In order to get plants representing all characters such as early and late, fine and coarse-grained, awned and awnless, white and red-kernelled, etc., a fairly wide selection of varieties was made. Although the chief cultivated types may differ considerably from one another the outstanding differences are exhibited by extreme forms like *sathi* and the wild rices (*pasai*) which have also been included in this study. Altogether twenty-five strains possessing diverse characteristics were examined but results of only twelve of the more important types are enumerated in this paper.

For investigating the chromosome complement of each plant both anthers and root tips were used. The material was obtained from the genetically pure strains growing in the field. In collecting suitable material, a preliminary examination of the anthers by Belling's [1921] iron-aceto-carmine method was necessary. The required phase for chromosome counts in the pollen mother cells was found in florets with thin, papery, pale-coloured inner glumes while the panicle was still completely enclosed in the leaf-sheath.

Fixing was restricted to bright mornings from eight to eleven o'clock during which period meiotic divisions were found to be most active. A large variety of fixatives was tried. On the whole either Navashin's fixative [1927] or Allen's modification of Bouin [Lee, 1928] solution, each used after dipping the material for about one minute in Carnoy's fluid [1901] gave better results in comparison to La Cour's 2BE fixative [1931] and Flemming's weak solution [1901]. Flemming's solution modified [1932] gave excellent preparations. It had an advantage over other fixatives for it gave good results even without a preliminary dip of the material in Carnoy's fluid . Flemming's modified was particularly good for the late prophasic stages and other subsequent stages while Navashin's solution, when

PLATE LIX


1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17

used after Carnoy's fluid does not give threads of such sharp definition as does Allen's Bouin. Threads in the former case become more granular and are therefore difficult to trace with certainty. The usual methods of dehydration, infiltration and embedding were adopted. The best sections were those cut at a thickness of about 12 μ. Staining was mostly done with Haidenhain's iron-alum haematoxylin and by Newton's [1929] iodine-gentian-violet method. Better results were obtained by the latter process, particularly so when the material was fixed with Navashin's solution.

Root tips were fixed between two and five o'clock on bright afternoons. The best results were obtained either with modified Flemming's solution or Navashin's solution. In this case 8 μ thick sections gave the best results.

III. CYTOLOGICAL OBSERVATIONS

Type 26.—It is popularly known as *anjee* in Basti district ; is a medium fine-grained, late-ripening and non-lodging variety..

Gametic complement (n=*12*) : The pre-meiotic divisions in the sporogenous cells and the various prophasic stages are quite normal. Plate LIX, fig. 1 shows the commencement of the prophase. Frequently, in well-fixed material, a beaded appearance in the threads could be observed (Plate LIX, fig. 2) which was more pronounced towards the periphery of the nucleus. This beaded structure suggests that the chromosomes are made up of strings of granules which are only seen when thickened by material from anastomosis. Hedayetullah [1931] in Narcissus has shown that the somatic chromosomes consist of two parallel threads which during prophase appear like strings of granules arranged in the form of chromosomes. By careful examination he found that anastomosis between the threads connected adjacent granules. Plate LIX, fig. 3 depicts similar connections in T 26.

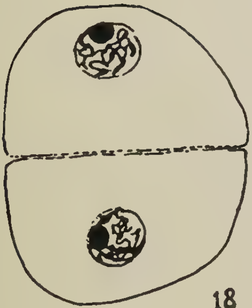
Parallel approximation of the spireme becomes more prominent in the post-synizetic stage when the nucleolus lies flattened against the nuclear membrane (Plate LIX, fig. 3). A little later the spireme appears as twelve loops scattered throughout the nucleus, each representing a bivalent chromosome (Plate LIX, fig. 4). The synaptic mates in Plate LIX, fig. 4 are observed twisted round each other. The loops slowly get shorter and thicker and the association of the parasynaptic mates becomes more intimate (Plate LIX, fig. 5). As a result of continued shortening and thickening the chromosomes attain a compact form. This leads to diakinesis. Pairing of the chromosomes at diakinesis is regular and the number of bivalents is always twelve (Plate LIX, fig. 6).

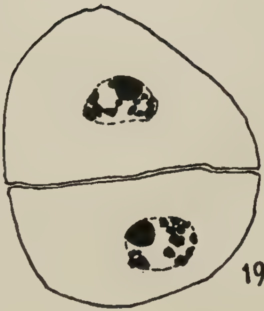
The nucleolus size is gradually diminished and its staining power is decreased as late diakinesis dwindles into early metaphase, at which stage the nucleolus entirely disappears (Plate LIX, figs. 6-9). Occasionally, on the nucleolus at prophase and at the end of interkinesis one or more chromatic bodies were noticed. Such chromatic structures in lentils were presumed to be nucleosomes by Miranda, Faustino [1931]. The number, size and occurrence of such structures was not found to be constant in the case of *Oryza sativa*. In certain cases two nucleosomes were observed, one larger than the other (Plate LIX, fig. 7). Sometimes, however, only one nucleosome could be seen while, more generally, the nucleolus had no nucleosome. Similar chromatic structures were observed by Kuwada [1910] who called them dwarf-nucleoli in his material of *O. sativa*. These structures may even be the same as the endonucleoli found by Verbrugge [1934] in some material of *Oenothera rubicalyx* and *O. blandina*. Verbrugge [1934], in his *Oenothera* material, noticed that the threads in rare cases were connected to the nucleolus by means of the endonucleolus. Only one case of such a connection was observed in *O. sativa* (Plate LIX, fig. 1). In one pollen mother cell Verbrugge noticed a nucleolus in which the endonucleolus appeared to be divided. Whether the occurrence of two or more nucleosomes in *O. sativa* is due to a similar division or have had separate origins is not known.

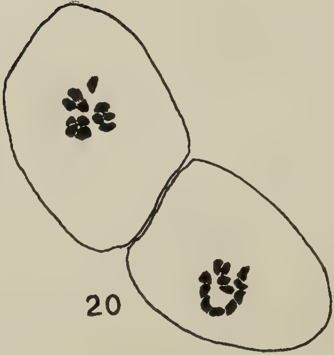
Generally, in prophase the chromosomes show variation in size and shape but in *O. sativa* the chromosomes are so small that they do not lend themselves to a detailed comparison. In certain cases the chromosomes were observed arranged in groups. Plate LIX, figs. 6-8 show such an arrangement. The chromosomes gradually become grouped at the centre of the nucleus which leads to metaphase of the first division (Plate LIX, figs. 9-11). With the advance of metaphase the bivalents attain a well-organised and smoother outline till they orient themselves on the equatorial plate of the spindle (Plate LIX, figs. 10 and 11).

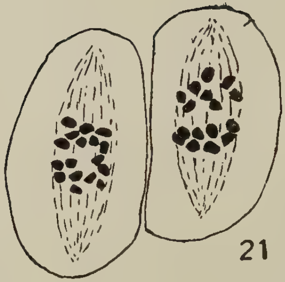
A bipolar spindle, which has been in the course of formation since late diakinesis, now appears at early metaphase (Plate LIX, fig. 12). Kuwada [1910] maintains that in *O. sativa* a tripolar spindle is first formed which later on assumes a

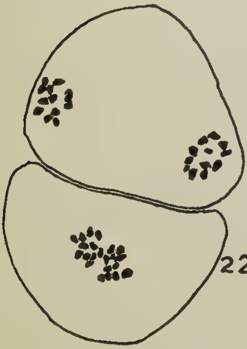
PLATE LX

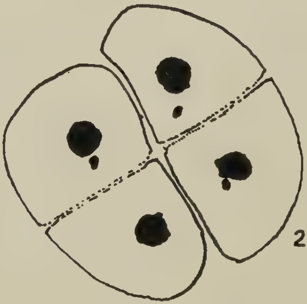

18

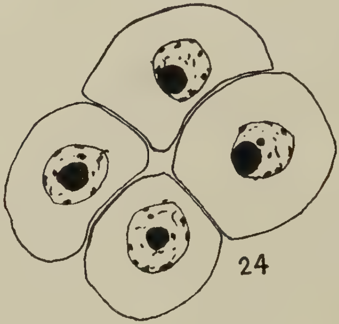

19

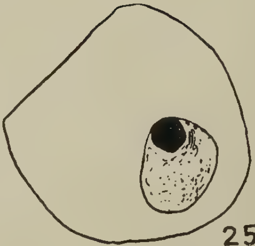

20

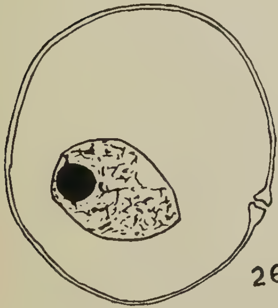

21

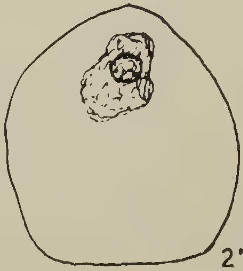

22

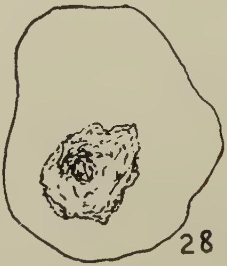

23

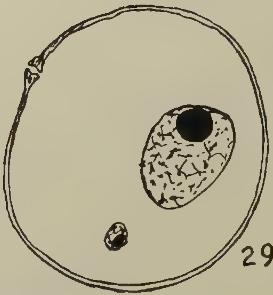

24

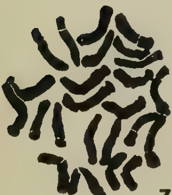

25

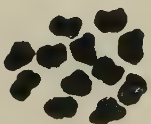

26

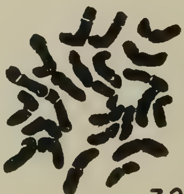

27

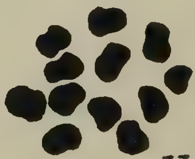

28

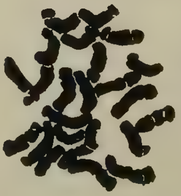

29

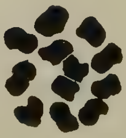

30


31


32


33


34


35

(*For explanation see page 705*)

bipolar shape. No tripolar spindle was observed in the course of this study. It is also believed that multipolar appearance of the spindle and extra fibres surrounding the structure may also arise on account of inadequate fixation. Some of the spindle fibres can be seen extending to the poles from definite points on the chromosomes (Plate LIX, fig. 12). Others which are only observed during anaphase and telophase are as a rule less prominent and show no connection with the chromosomes. Chromosomes now look simpler in form with minimum degree of bivalency (Plate LIX, fig. 11). Twelve bivalents could be very easily counted at this stage. Lagging of one or two bivalents was often observed during anaphase. The homologues may appear to lag either on account of delayed disjunction of the bivalents or due to their detachment from the spindle fibres. Plate LIX, fig. 15 depicts the two halves of the lagging bivalent still in the course of migration to the poles while their comrades have reached the poles and started the second division. Sometimes, however, the laggards separate soon enough so that the homologous parts reach the poles in time for participating in the second mitosis (Plate LIX, fig. 14). Normal daughter nuclei will thus be obtained. Often a deficiency in the constitution of the daughter nuclei is secured through the exclusion of the laggards. In certain cases only one of the separated halves is included, while the other is seen on the spindle (Plate LIX, fig. 16). Still in other cases both homologues may be included in the same daughter nucleus, or both of them may remain extruded and form supernumerary nuclei. Thus the possibility arises, favouring the formation of dissimilar daughter nuclei. In Plate LIX, fig. 14 the chromosomes are seen forming a clump at each pole of the spindle. Soon this mass loses the power to take up stains. A nuclear membrane then surrounds the chromosomes which lose their identity (Plate LIX, figs. 14, 16 and 17). This leads to the second mitosis. Then a large nucleolus appears (Plate LX, fig. 18). Complete disintegration of the chromosomes before the commencement of the homotypic divisions and the formation of the secondary nucleolus shows a long interkinesis period. A continuous spireme is formed in the daughter nuclei at interphase (Plate LX, fig. 18). A reticulum, however, is not formed but the spireme breaks up transversely to give twelve chromosomes (Plate LX, fig. 19).

In a polar view of homotypic metaphase (Plate LX, fig. 20) twelve chromosomes can be counted but curiously enough they present a paired arrangement which is generally observed in a diploid cell and not in the course of an ordinary homotypic division or in a haploid cell. This peculiar behaviour of chromosomes in homotypic division was first discovered in *Morus* by Tahara [1910] and its occurrence in *O. sativa* was established by Kuwada [1910]. The present observations are therefore in agreement with those of Kuwada. Pairing, however, was not noticed in all the twelve chromosomes, some remained single while others were observed in groups of two or three. The arrangement of the chromosomes of the two sister nuclei in the homotypic division, is generally identical but not invariably so. The biological significance of this pairing of chromosomes is not yet explored. Sometimes the two sister cells start homotypic divisions simultaneously (Plate LX, fig. 21) but more frequently the divisions commence in one of the nuclei before the other, so that the two daughter nuclei can be seen at different developmental stages (Plate LX, fig. 22). Plate LX, fig. 24 shows a pollen mother cell in the tetrad stage. When the tetrad cells separate they tend to retain, for some time, the characteristic shape found at the tetrad stage (Plate LX, fig. 25). As a result of the irregularities in the first meiotic mitosis the tetrad nuclei show corresponding differences, thereby causing dissimilarity in the pollen grain constitution (Plate LX, fig. 23). Some of the pollen grains appear to be in a state of degeneration (Plate LX, figs. 27 and 28); their cytoplasm seems thin and highly vacuolated and the cells are irregular in outline. Their nuclei vary in size and have a poorly organised appearance. These may be regarded as the n—1 type of microspores in which case the complement is short of one chromosome and the deficiency in the chromatic substance perhaps is responsible for degeneration. Still others present unusual conditions in possessing more than one nucleus. The multinucleate type is formed by the presence of the supernumerary nuclei (Plate LX, fig. 29). Some pollen may even contain $n+1$ number of chromosomes. While a large number of morphologically perfect pollen grains, quite normal in constitution, is also met with (Plate LX, fig. 26).

During the course of the above investigation, a noticeable variation with regard to the phases of meiosis in the anthers of the same flower were observed. One anther may have pollen mother cells showing metaphase while in another only the early prophasic stages may be seen ; still others may show the second division stages. Again, the apical end of an anther as a rule showed more advanced stages than the basal end. It is apparent, therefore, that in a particular spikelet the meiosis starts at different times in the anthers and, further, that the meiosis is initiated first at the top of the loculus so that the lower portion will always be slightly behind in development. When the pollen mother cells are in the prophase stage the colour of the anthers is white. During successive stages of meiosis the anthers turn pale yellow and at full growth of pollen they attain a yellowish colour. The size of the anthers is about 1 mm. when the sporogenous tissue can be distinguished.

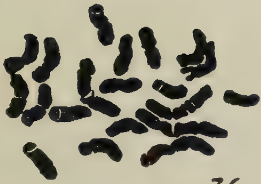
Somatic complement (*2* n=*24*). Somatic mitosis was observed to be quite normal. At metaphase the chromosomes are generally very crowded on the plate, owing to their large size relative to the nucleus. In clear plates, however, twenty-four chromosomes were counted (Plate LX, fig. 30). With the small and contorted nature of the chromosomes and the constrictions vaguely shown, only a very careful examination of the plates could locate the place of constriction. Chromosomes showed either terminal, sub-terminal, median or sub-median constrictions. Sometimes the constricted part may become so drawn out that the two portions look separated. This causes confusion in making accurate computations. More often a constriction is denoted by mere depression in the contour of the chromosome. Both types of constrictions were observed. As regards size, the somatic chromosomes could be divided into three groups namely, large, medium and small. In T26 (Plate LX, fig. 30) it was possible to make out eight pairs of large, two pairs of medium and two pairs of small chromosomes. The length of chromosomes in the entire complement varies from $1 \cdot 7\mu$ to $2 \cdot 5\mu$. The spindle fibres seem to be attached to the chromosomes at the constricted points. Trabants were not observed. They are either absent or too small to be visible. In comparison to other species studied, somatic chromosomes in T 26 appear more slender, longish and less curved. The homologues lie more closely in approximation than what is found in the other types. In respect of shape and position of constrictions, the chromosomes can be classified as shown in Table I.

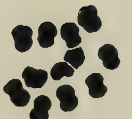
Cytological observations on other paddy varieties are briefly enumerated in Table I.

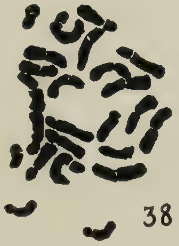
TABLE

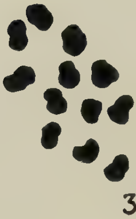
Summary of

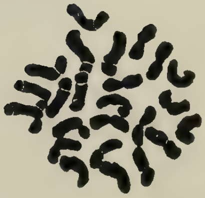
Variety	Popular name	District of origin	Distinguishing characters	Gametic complement	
				Meiosis	No. of bivalents
T 2	*Safed munji.*	Saharanpur.	Early, awnless, fine grain, light purple outer glumes and red purple apiculus, outer glume colour fades away near ripening.	Irregular, 1 or 2 bivalents lag behind but often these disjoin in time for participating in the second mitosis.	12, Plate LX, Fig. 31.
T 3	*Bansmati*	Dehra Dun	Medium-early, awnless, fine-grain, very fragrant, white outer glumes and deep yellow apiculus.	Regular, no lagging . .	12, Plate LX, Fig. 33.
T 5	*Hansraj*	Gonda .	Very late, awnless, fine to bold-fine grain and white outer glumes.	Fairly regular, one bivalent showing tendency towards lagging but participates in second mitosis.	12, Plate LX, Fig. 35.
T 21	*Chawl* .	Rampur State.	Medium-early, awnless, medium-bold fine grain.	Irregular, 2 or 3 bivalents lag but mostly these participate in II mitosis. Occasionally one bivalent remained excluded causing dissimilar pollen grains.	12, Plate LXI, Fig. 37.
T 23	*Kalasukhdas.*	Banda .	Late, fragrant, fine to bold-fine grain, purple outer glumes, dull orange inner glumes, and short awns of orange brown colour.	Regular, bivalents show pronounced bivalency and smaller in size than those of others except *Pasai* and T 21.	12, Plate LXI, Fig. 39.
T 26	*Anjee* .	Basti .	Late, awnless, medium-fine grain	Irregular, sometimes producing dissimilar pollen.	12, Plate LIX, Fig. 11.

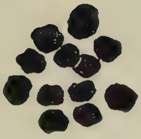

36

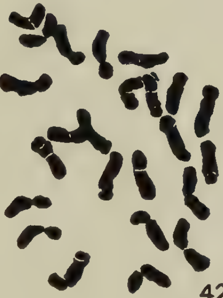

37

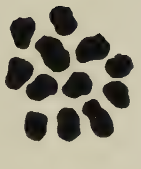

38

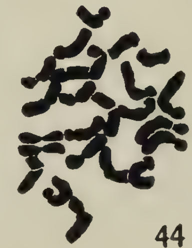

39

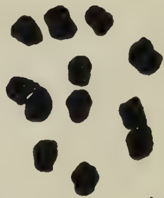

40

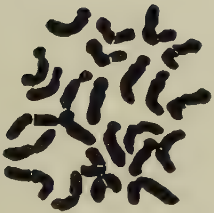

41

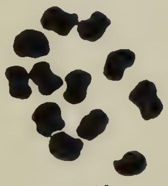

42

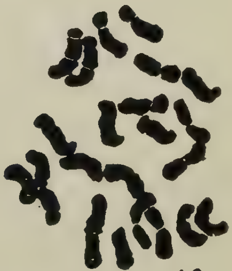

43

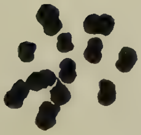

44

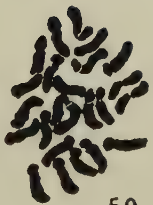

45

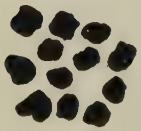

46

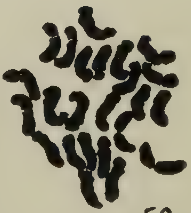

47


48


49


50


51


52

(*For explanation see page 706*)

I

cytological observations

Somatic complement													
Size of chromosomes			Chromosome length in μ		Location of attachment constrictions							Total No. of 2*n* chromosomes	Morphological description
									Double constrictions				
Large	Medium	Small	Minimum	Maximum	Terminal	Sub-terminal	Sub-median	Median	Ter. and sub-median	Ter. and median	Subter. and median		
12	8	4	1·4	2·2	2	8	6	8	...	...	...	24 Plate LX, fig. 32.	J- and L-shaped chromosomes present with subterminal constrictions on the longer arms.
10	8	6	1·4	2·1	6	8	2	2	2	4	...	24 Plate LX, fig. 34.	Chromosomes are thicker and more compactly arranged when compared with those of T 2; size differences not well demarcated.
8	12	4	1·2	2	6	2	8	4	...	4	...	24 Plate LXI, fig. 36.	Chromosomes well spread out and thicker in comparison to T 2 and T 3; L-, J-, and V- shaped chromosomes not found.
10	8	6	1·1	2·2	2	6	8	6	...	...	2	24 Plate LXI, fig. 38.	The presence of two U-shaped submedianly constricted chromosomes is unusual. Chromosomes least bulky when compared with the other varieties except those found in *pasai*. The smallest pair here is the shortest of all the pairs in other varieties. Largest range of chromosome length.
6	10	8	1·7	2·1	2	6	8	6	2	...	...	24 Plate LXI, fig. 40.	Two pairs of L-shaped chromosomes present; one of these is submedianly and terminally constricted while the other pair has only submedian constrictions. Two somewhat S-shaped chromosomes are observed. Comparatively shorter and less bulky complement.
16	4	4	1·7	2·5	4	8	6	2	2	2	...	24 Plate LX, fig. 30.	Chromosomes comparatively thin and longish. V- and L-shaped chromosomes are present, the former being submedianly constricted with the construction at the bend. L-shaped pair is both submedianly and terminally constricted with the sub-median constriction at the bend and the other located on the longer arm.

TABLE

Summary of

Variety	Popular name	District of origin	Distinguishing characters	Gametic complement	
				Meiosis	No. of bivalents
T 75	*Gunchchai*	Shahjahanpur.	Late, awnless, medium-coarse grain, white outer glumes and whorled arrangement of spikelets.	Regular	12, Plate LXI, Fig. 41.
T 94	*Kaladhan*	Bombay Presidency.	Late, awnless, bold-coarse grain dull orange inner glumes, purple apiculus and deep purple colour of leaves.	Regular	12, Plate LXI, Fig. 43.
T 110	*Ramjai* .	Allahabad	Late, awnless, short roundish grain, light red outer glumes, brownish inner glumes and red purple apiculus at maturity.	Lagging observed but laggards participate in II mitosis. Chromosomes bigger than other late varieties.	12, Plate LXI, Fig. 45.
T 131	*Sathi* .	Gazipur	Very early, short awns, coarse grain, black inner glumes, red kernel, red purple apiculus, enclosure of the panicle within sheath, grains shed at maturity and very short plant height.	Irregular, resulting in supernumerary nuclei.	12, Plate LXI, Fig. 47.
Wild rice.	*Pasai* .	Nagina .	Medium-early, very long awns, medium-coarse grain, dull black inner glumes, plant habit spreading and grain shedding.	Regular, two bivalents unusually small.	12, Plate LXI, Fig. 49.
Italian paddy.	*Frecoce allorio*	Italy .	Early, awnless, coarse grain, light orange inner glumes and short stature of plant.	Lagging observed but laggards participate in second mitosis.	12, Plate LXI, Fig. 51.

I—*concld.*

cytological observations—concld.

Somatic complement													
Size of chromosomes			Chromosome length in μ		Location of attachment constrictions							Total No. of $2n$ chromosomes	Morphological description
									Double constrictions				
Large	Medium	Small	Minimum	Maximum	Terminal	Sub-terminal	Sub-median	Median	Ter. and sub-median	Ter. and median	Subter. and median		
10	8	6	1·7	2·5	4	2	10	4	4	...	...	24 Plate LXI, fig. 42.	Well spread out, thick and less curved chromosomes. One L-shaped pair like T 26 with both terminal and submedian constrictions.
10	10	4	1·4	2·5	2	8	10	4	...	...	...	24 Plate LXI, fig. 44.	L- and U-shaped chromosomes present. U-shaped with terminal constrictions; one of the L-shaped pairs is similar to the submedianly constricted L-shaped pair of T 23.
14	6	4	1·7	2·7	2	6	6	8	...	2	...	24 Plate LXI, fig. 46.	Two pairs of L-shaped chromosomes and one pair of V-shaped chromosomes are outstanding. One L-shaped pair with median constrictions and the other is sub-medianly constricted. A sub-median constriction is also found on the V-shaped pair. One pair somewhat U-shaped with median constrictions. Possesses largest length of the smallest chromosome in comparison to other varieties.
10	8	6	1·5	2·7	4	4	8	6	2	...	...	24 Plate LXI, fig. 48.	Very thick and sparsely arranged chromosomes at heterotypic metaphase. Contours very irregular and size differences less marked.
8	14	2	1·2	1·7	2	12	8	2	...	...	...	24 Plate LXI, fig. 50.	L- and J-shaped chromosomes are found with submedian and terminal constrictions respectively. Chromosomes smallest in bulk, possessing a minimum range in length. Constrictions inconspicuous. Size differences not pronounced.
14	6	4	1·5	2·1	4	8	8	4	...	...	...	24 Plate LXI, fig. 51.	J-shaped chromosomes with subterminal constrictions present. Individual differences in chromosome lengths and constrictions not well pronounced. U- and L-shaped chromosomes absent.

IV. Discussion

Unlike majority of plants in Gramineae, the genus *Oryza* is characterised by twelve as its basic number of chromosomes. Chao [1928] in his studies on the glutinous gene in rice described No. 100 as diploid and Nos. 200 and 1300 as the tetraploid forms. Scented non-glutinous Chinese variety corresponding to No. 1000 was stated to possess twenty-four somatic chromosomes by Morinaga and Fukishima [1931]. Chao, however, does not specify the chromosome number of the tetraploid varieties. Further, the grouping of chromosomes in the homotypic metaphase as observed by Kuwada [1910] and the paired arrangement of chromosomes in the somatic metaphase led Lawrence [1931] to think the variety to be a secondary polyploid. On account of the homology that exists in the chromosomes and considering the presence of some duplicate factors, Lawrence suggests that the species has been derived from a form with seven pairs of chromosomes. Morinaga and Fukishima [1931] contrary to Kuwada did not notice paired arrangement of chromosomes in somatic metaphase. Tendency for association of chromosomes in the homotypic metaphase was however observed but no such grouping was found in heterotypic metaphase. The results of the present investigation are entirely in agreement with those of Kuwada, for both grouping at homotypic metaphase and paired arrangement of chromosomes in somatic metaphase were observed. Further, there is sometimes evidence of slight tendency towards grouping of the chromosomes even at diakinesis. Species in the sub-families closely related to *Oryzeae* contain five or seven chromosomes. *Oryza sativa* therefore can be regarded as a secondary polyploid which has originated through the initial natural hybridization of the five and seven chromosome types.

In respect of chromosome numbers, the results of the present investigation are in agreement with those of other workers, namely, Kuwada [1910], Nakatomi [1923], Kato [1930], Selim [1930], Morinaga and Fukishima [1931] and Ramiah [1932]. There are each in T 3 and T 21 five large pairs, four medium and three pairs of small chromosomes in which respect the results are consistent with those obtained by Rau [1929] in his material of *O. sativa*. Rau [1929], however, states that the size of the large chromosomes is double of the small ones which is not quite so in T 3 ; the largest pair being about 2·1μ in length and the smallest about 1·4μ but holds good for the results pertaining to T 21. It is also clear from this vast study of varieties that all forms of *Oryza sativa* are identical in chromosome numbers irrespective of their diverse characteristics. Even the extreme forms like *sathi* and *pasai* show no variation in number from other types. The differences in their external characters are therefore entirely due to certain differences in the constitution of chromosomes. It has been possible to classify the somatic chromosomes in respect of their morphological differences. Cytology has been frequently employed in attempts for illucidating plant relationships. Unfortunately, rices do not afford a particularly favourable material for the purpose as the nuclei are small and the chromosomes are fairly uniform in shape and size.

The varieties, although consistent in chromosome numbers, differ to a certain extent in the morphology of their chromosomes. Chromosome bulk in T 131 is greater in comparison to other varieties. The size of the chromosomes although varies amongst the different types, the differences are not large enough for lending any significant comparison. According to Lesley and Frost [1927] and also Darlington [1929] the chromosome size and structure may be governed by genes. Such variations in the present case may, therefore, be due to the diversity of genes. The somatic complements of *pasai* and *sathi* exhibit maximum difference with chromosomes ranging in length from $1\cdot2\mu$ to $1\cdot7\mu$ and $1\cdot7\mu$ to $2\cdot7\mu$ respectively. Whether such slight differences, as are observed here, are of any significance is a matter yet to be considered. Peto [1933] in his cytological studies of *Festuca-Lolium* hybrids regards a difference of about $1\cdot5\mu$ to be insignificant, for according to him such variations may even be observed in different cells of the same root tip. The difference in the present case is even less, maximum being 1μ only. Further, as Babcock [1931] points out, specific differences are not necessarily accompanied by definite chromosome variation ; specific differentiation is more often effected by an accumulation of genic differences, having no visible change on gross chromosome structure. The position on the whole in respect of the present results, therefore, is such that it calls for further investigation before definite conclusions can be drawn.

It is also evinced that certain chromosomes, despite their similarity in shape and form, differ with regard to the nature of attachment constrictions as well as in length. T 2, for instance, is marked by the presence of one pair of L-shaped and another pair of J-shaped chromosomes ; both possess sub-terminal constrictions on the longer arms. The two L-shaped pairs of T 23, although identical in size to that found in T 2, vary regarding the location of their constrictions, one being sub-medianly constricted and the other bears both terminal and sub-median constrictions. The former pair, however, is somewhat similar to one of the two L-shaped pairs of T 110 while the latter corresponds to the L-shaped pair observed in T 75. It is also similar to the L-shaped pair of T 26 with regard to the position of attachment constrictions but differs in length which seems to be greater in T 26. The other L-shaped pair of T 110 is both terminally and medianly constricted which in respect of the attachment constrictions is quite unique and characteristic of the variety. Again, out of the two L-shaped pairs of T 94, the larger one is similar in size and shape to those of T 2, T 23 and T 75 but differs in the location of attachment constrictions which are terminal in T 94, sub-terminal in T 2, sub-median in T 23 and both terminal and sub-median in T 75. The smaller L-shaped pair of T 94 corresponds to the sub-medianly constricted L-shaped pair of T 23, T 110 and *pasai* but differs in size. Although the results do not afford enough material, so as to enable specific ranking of the varieties, the similarity amongst the chromosome complexes of the varieties indicates that the plants concerned must be identical in certain characters which assertion finds confirmation from the comparison of their external characters. Even on these results, therefore, it is quite safe to predict that the varieties will have certain features in common and their grouping together in respect of these is quite justified. The presence of somewhat S-shaped pair, along with other morphological differences like size of chromosomes, demarcate T 23 from T 110. Whereas T 21, T 94, T 110 can be linked, to a certain extent, on account of the presence of U-shaped chromosomes variations concerning size and location of attachment constrictions of these chromosomes reveal that the complements of the three strains are not quite synonymous. The presence of U-shaped chromosomes and other morphological details pertaining to T 94, a late variety, at once separate it from other late types like T5, T23, T26 and T 75. Further, T 26 resembles T 110 in the presence of V-shaped chromosomes, which in T 26 are comparatively longer. *Frecoce allorio* (Italian paddy) tallys with T 2 in respect of the J-shaped chromosomes. J-shaped chromosomes in *pasai*, however, are terminally constricted on the shorter arms ; these therefore differ from those of T 2 and *Frecoce allorio* in which case the chromosomes bear sub-terminal constrictions on the longer arms. T 110 and T 3 too have somewhat V-shaped chromosomes with arms widely apart. The differences in the morphological characters indicate that the chromosomes of the various types are unlike ; yet a certain amount of similarity does exist amongst their complements. Again, the existence of intermediate forms between the extreme types suggests that chromosome constitution does not differ greatly. From a consideration of the morphological details, it is quite possible to assume that various strains of rices included in this study, although consistent in certain respects, show a fair degree of inconsistency as well, so that the different strains can be regarded as different forms of the same species. The variability, however, is not so great for regarding them as distinct species. The results therefore are worthy of lending some support for linking certain varieties and further they suggest that more critical investigations concerning structural composition of chromosomes will supply material for adequate grouping of various strains which will ultimately throw light upon their taxonomic relationships.

In *pasai* despite the general diminutive size of the entire gametic complement, two bivalents are markedly small in size and therefore characterise the variety. Upon similar observations Kuwada [1910] is inclined to think a plant of *Oryza sativa* to be abnormal and of mutant origin. Beside the presence of these two unusually small bivalents, there is no other evidence available in this investigation in support of the mutant origin of *pasai*.

The varieties can also be grouped in respect of the behaviour of meiotic chromosomes. In T 3, T 75, *pasai* and *Frecoce allorio* the bivalents behave regularly at meiosis, whereas T 5, T 21, T 23 and T 94 are marked by the lagging of one or two bivalents at the equatorial plate. The laggards, however, disjoin in time so as to reach the spindle poles before the commencement of second division. Pollen, therefore, in spite of this irregularity, can be expected to be quite normal.

More serious irregularities are observed in T 2, T 26, T 110 and T 131, in which group, occasionally, the laggards are not included in the daughter nuclei but remain excluded and form supernumerary nuclei. Due to such irregularities many of the resulting pollen grains must receive a genetically unbalanced complement of chromosomes. If the pollen with unbalanced set of chromosomes be morphologically fit for fertilisation, it becomes quite possible to expect, the existence or origin of plants possessing chromosome numbers different from the normal. These might arise through the union of $n-1$ or $n+1$ with normal gametes. If two such $n-1$ or $n+1$ gametes unite, the resulting plant will have twenty-two or twenty-six chromosomes respectively. Since no such plants have been observed so far, it may safely be assumed that gametes having $n-1$ or $n+1$ chromosome numbers are physiologically imperfect ; hence incapable to effect fertilization. Frequently, degenerated pollen grains were also observed. It is possible to speculate that these are the $n-1$ forms which on account of the chromatic deficiency start degenerating. Both the degenerated and multinucleate pollen grains are morphologically sterile. It is, therefore, not surprising that a low percentage of pollen appears to be normal within this group. The sterility percentage* of the group ranges from seventeen to forty and the abundance of sterile spikelets in these strains may be due to the imperfect pollen grains. The sterility percentage of the group where the bivalents are included in second mitosis is about ten, and where no irregularities are observed the sterility percentage is about six only. Defective pollen may even be due to inadequate food supply which interferes with the reduction divisions. Bad pollen has also of late been associated with irregular meiosis which is thought to indicate hybridism. It seems perhaps unwise to assign any single cause to defective and abortive pollen and other abnormalities that are associated at times with microsporogenesis. Rosenberg's [1909] investigations of a natural *Drosera* hybrid demonstrated a close correlation between meiotic irregularities and the production of sterile pollen. Pronounced sterility besides the environmental effects, therefore, may primarily be the result of more serious meiotic irregularities that exist in the group.

More extended researches by several workers on a number of genera, widely separated taxonomically, have shown such a close correlation between meiotic irregularities and known and suspected hybrid forms that the presence of such irregularities in a species has come to be regarded as strong evidence for its origin through hybridization. According to Ernst [1918] hybridization is the initial cause of meiotic irregularities, apomixis, polyembryony, etc. It is, therefore, possible to conceive that varieties like T 3, T 75, *pasai* and *Frecoce allorio*, showing regular meiosis had an earlier existence and those with lagging chromosomes like T 5, T21, T23, and T94 have subsequently originated from the already existing forms through natural crossing and became stable varieties in the course of a very long period. These, therefore, may be considered to have had a later existence in this world. More recent origin is of T 2, T 26, T 110 and T 131 which show serious irregularities. Cytological possibilities for the origin of new rice varieties through natural crossing lie in the fact that the chromosomes of various types are relatively similar, and further, all the types are consistent in their chromosome numbers. Both these factors render hybridization easy. The genetical evidence, however, consists in the ease with which divergent rice varieties can be successfully crossed, giving no indication of any serious degree of sterility in the hybrids. The possibility of natural hybridization is also increased when the varieties are endemic in the same locality. This is what we find in the case of rice varieties.

---

* Sterility percentage for each variety was worked out on five ears' produce only as enough material was not available in the laboratory at the time this information was suddenly required.

V. Summary

1. A number of varieties representing diverse characters have been studied in respect of their chromosome numbers, morphology of chromosomes and their behaviour at meiosis.

2. Despite great diversity in plants and the wide geographical range dealt with, all forms are consistent in chromosome numbers, possessing twelve and twenty-four as their haploid and diploid chromosomes respectively.

3. Unlike majority of plants in Gramineae, *Oryza sativa* is characterised by twelve as its basic number of chromosomes.

4. The grouping of chromosomes at homotypic metaphase is unusual. Such associations were also occasionally observed in the diakinesis stage. Paired arrangement of chromosomes in somatic metaphase was quite apparent.

5. *Oryza sativa* can be regarded as a secondary polyploid having originated through the natural hybridization of species in the sub-families closely related to *Oryzeae*, possessing five and seven chromosomes.

6. The complements of different types show definite variation in chromosome morphology. Chromosome constitution therefore is responsible for the varietal differences exhibited for each type.

7. Somatic complements have been analysed in respect of their morphological details.

8. T 2, T 23, T 26, T 75, T 94, T 110 and *pasai* are characterised by the presence of L-shaped chromosomes and T 21, T 94 and T 110 can be linked to a certain extent for possessing U-shaped chromosomes. U-shaped chromosomes and other morphological details of T 94, a late variety, separate it from other late types like T 5, T 23, T 26 and T 75.

9. The strains can be regarded as different forms of the same species. The variability is not so great for classifying them as distinct species.

10. Varieties are also grouped in respect of the behaviour of meiotic chromosomes. In T 3, T 75, *pasai* and *Frecoce allorio*, the bivalents behave regularly at meiosis whereas T 5, T 21, T 23 and T 94 are marked by the lagging of one or two bivalents which, however, are included in the daughter nuclei. In T 2, T 26, T 110 and T 131, the diad nuclei receive genetically unbalanced complement of chromosomes causing diversity in pollen grain constitution. More pollen grains in this group are physiologically sterile.

11. A positive correlation exists between meiotic irregularities and sterility.

12. It is possible to conceive that varieties like T 3, T 75, *pasai* and *Frecoce allorio* had an earlier existence in this world, whereas T 5, T 21, T 23 and T 94 originated subsequently from the existing forms through natural crossing ; more recent origin is of T 2, T 26, T 110 and T 131 showing serious irregularities.

The study was undertaken as a part of the Rice Research Scheme in the United Provinces, financed by the Imperial Council of Agricultural Research to whom the writer is greatly indebted for providing necessary facilities. The writer further wishes to thank Rai Bahadur R. L. Sethi, I.A.S., Economic Botanist to Government, U. P., under whose control the work was conducted, for helpful suggestions and the keen interest he took in the work. Thanks are also due to Mr. T. R. Mehta for aid in the preparation of the photographs.

REFERENCES

Babcock, E. B. (1931). *Amer. Nat.* **65**, 5-18.

Belling, J. (1921). *Amer. Nat.* **55**, 573-574.

Chamberlain, C. J. (1901). 'Methods in Plant Histology', p. 327.

Chao, L. F. (1928). *Gen.* **13**, 191-225.

Darlington, C. D. (1929). *J. Gen.* **21**, 207-286.

Ernst, A. (1918). 'Bastardierung als Ursache der Apogamies im Pflanzenreich'. Jena.

Huskins, C. L. (1927). *J. Gen.* **18**, 315-364.

Jenkin, T. J. and Sethi, B. L. (1932). *J. Gen.* **28**, 1-36.

Kato, S. (1930). *J. Dept. Agric. Kyushu Imp. Univ.* **2**, 241-276.

Kuwada, Y. (1910). *Bot. Mag. Tokyo* **24**, 267-281.

La Cour, L. (1929). *Nature*, 124-127.

Lawrence, W. J. C. (1931). *Cytologia* **2**, 354-384.

Lee, B. (1928). 'Microtomist's Vade-Mecum.'

Lesley, M. M. and Frost, H. B. (1927). *Gen.* **12**, 449-460.

Miranda, Faustino (1931). *Bol. Soc. Espan. Hist. Nat.* **31** (6), 403-407.

Morinaga, T. and Fukishima, E. (1931). *Bot. Mag. Tokyo* **45**, 140-145.

Nakatomi, S. (1923). *Jap. J. Gen.* **2**, 107-115.

Newton, W. C. F. and Darlington, C. D. (1929). *J. Gen.* **21**, 1-15.

Navashin, M. S. (1927). *Bull. Ann. Bot. Gen. and Plant Breeding* **17**, 147-150.
Peto, F. H. (1933). *J. Gen.* **28**, 113-156.
Ramiah, K. (1932). *Current Sci.* **6**, 166.
Rau, N. S. (1929). *J. Ind. Bot. Soc.* **8** (3), 201-206.
Rosenberg, O. (1909). *Kgl. Svensk. Vet. Handl.* **43**, 3-64.
Selim, A. G. (1930). *Cytologia* **2**, 1-26.
Tahara, M. (1910). *Bot. Mag. Tokyo* **24**, 281-289.
Verbrugge, M. (1934). *Ann. Bot.* **48**, 189, 153-177.

EXPLANATION OF PLATES

[Drawings were made with the aid of an Abbé camera lucida, employing a Zeiss apochromatic 1·3 mm. objective in combination with compensating eyepieces of various magnifications. The ×10 and ×20 eyepieces were more frequently used which gave magnifications of ×1800 and ×3600 respectively at bench level].

PLATE LIX

T 26

FIG. 1. Early prophase showing connection of spireme thread with endonucleolus (×1800).

FIG. 2. Showing beaded nature and parallel approximation of spireme (×1800).

FIG. 3. Pronounced approximation and beaded nature of spireme (×1800).

FIG. 4. 12 loops with the synaptic mates twisted round each other (×1800).

FIG. 5. Loops shortened and more intimate association of parasynaptic mates (×1800).

FIG. 6. Diakinesis showing 12 bivalent chromosomes with the nucleolus getting fainter (×1800).

FIG. 7. Diakinesis with a still fainter nucleolus and two nucleosomes; bivalents are seen in groups (×1800).

FIG. 8. Late diakinesis showing much fainter and smaller nucleolus, very thin nuclear membrane and thick blunt masses of bivalent chromosomes; bivalents are seen in groups (×1800).

FIG. 9. Gradual migration of the bivalents to the equatorial plate with nucleolus having disappeared and bivalents in groups (×1800).

FIG. 10. Heterotypic metaphase, polar view showing 12 bivalents arranged at the equatorial plate (×1800).

FIG. 11. Same as above, polar view magnified (×3600).

FIG. 12. Heterotypic metaphase, side view showing bipolar spindle and overlapping of chromosomes (×1800).

FIG. 13. Anaphase of first division showing 12 disjoined homologues migrating to the opposite poles (×1800).

FIG. 14. Telophase, with chromosomes losing identity; no laggards are seen (×1800).

FIG. 15. Both the halves of a lagging bivalent seen extruded at the end of first divisions (×1800).

FIG. 16. Only one of the halves is extruded, the other is probably included in second divisions (×1800).

FIG. 17. Widening of the phragmoplast and the chromosomes have lost their identity for the second divisions (×1800).

PLATE LX

FIG. 18. Commencement of second mitosis, showing a continuous spireme and a nucleolus in the diad cells (×1800).

FIG. 19. Late homotypic prophase showing 12 chromosomes (×1800).

FIG. 20. Homotypic metaphase, polar view showing 12 chromosomes arranged in each diad (×1800).

FIG. 21. Homotypic anaphase showing simultaneous stages in the diad cells (×1800).

FIG. 22. Homotypic divisions exhibiting different stages in the diads (×1800).

FIG. 23. End of second mitosis showing supernumerary nuclei (×1800).

FIG. 24. Tetrad stage (×1260).

FIG. 25. Pollen grain in an early stage (×1260).

FIG. 26. A normal pollen grain (×1260).

FIG. 27. Degenerated pollen grain (×1260).

FIG. 28. Same as above (×1260).

FIG. 29. A binucleated pollen grain (×1260).

FIG. 30. Metaphase in root tip of T 26, polar view showing 24 chromosomes (×3600).

FIG. 31. Heterotypic metaphase, polar view showing 12 bivalent chromosomes in the P. M. C. of T 2 (×3600).

FIG. 32. Metaphase in root tip of T 2 showing 24 chromosomes (×3600).

FIG. 33. Heterotypic metaphase showing 12 bivalent chromosomes in the P. M. C. of T 3 (×3600).

FIG. 34. Metaphase in root tip of T 3 showing 24 chromosomes (×3600).

FIG. 35. Heterotypic metaphase showing 12 bivalent chromosomes in the P. M. C. of T 5 (×3600).

PLATE LXI

FIG. 36. Metaphase in root tip of T 5 showing 24 chromosomes (×3600).

FIG. 37. Heterotypic metaphase showing 12 bivalent chromosomes in the P. M. C. of T 21 (×3600).

FIG. 38. Metaphase in root tip of T 21 showing 24 chromosomes (×3600).

FIG. 39. Heterotypic metaphase showing 12 bivalent chromosomes in the P. M. C. of T 23 (×3600).

FIG. 40. Metaphase in root tip of T 23 showing 24 chromosomes (×3600).

FIG. 41. Heterotypic metaphase showing 12 bivalent chromosomes in the P. M. C. of T 75 (×3600).

FIG. 42. Metaphase in root tip of T 75 showing 24 chromosomes (×3600).

FIG. 43. Heterotypic metaphase showing 12 bivalent chromosomes in the P. M. C. of T 94 (×3600).

FIG. 44. Metaphase in root tip of T 94 showing 24 chromosomes (×3600).

FIG. 45. Heterotypic metaphase showing 12 bivalent chromosomes in the P. M. C. of T 110 (×3600).

FIG. 46. Metaphase in root tip of T 110 showing 24 chromosomes (×3600).

FIG. 47. Heterotypic metaphase showing 12 bivalent chromosomes in the P. M. C. of T 131 (×3600).

FIG. 48. Metaphase in root tip of T 131 showing 24 chromosomes (×3600).

FIG. 49. Heterotypic metaphase showing 12 bivalent chromosomes in the P. M. C. of *pasai* (×3600).

FIG. 50. Metaphase in root tip of *pasai* showing 24 chromosomes (×3600).

FIG. 51. Heterotypic metaphase showing 12 bivalent chromosomes in the P. M. C. of *Frecoce allorio* (×3600).

FIG. 52. Metaphase in root tip of *Frecoce allorio* showing 24 chromosomes (×3600).

A NOTE ON THE CYTO-GENETICS OF *RICINUS COMMUNIS* L.

BY

R. H. RICHHARIA, Ph.D.

Agricultural Research Institute, Nagpur, Central Provinces

(Received for publication on 12th November 1936)

(With six text-figures)

CONTENTS

PAGE
I. Introduction 707
II. Material and technique 707
III. Meiosis 708
IV. Genetical data from literature 710
V. General consideration 710
VI. Summary 710
References 711

I. Introduction

As pointed out by White [1918], Harland [1920] and Peat [1928], the castor-oil plant, *Ricinus communis* L., is a satisfactory material for genetical analysis, for it exists in a large number of well-marked varieties which readily inter-cross and the seeds remain viable for several years. The hybridising technique is simple and the back-crosses for linkage studies are easily obtained. It is comparatively quick-growing and is free from serious pests and diseases. The haploid chromosome number being only ten, it should not be very difficult to establish linkage groups and chromosome maps. But the plants require enough space for their cultivation.

The seeds of the castor crop were collected here in 1934 by Mr. K. P. Shrivastava, Second Economic Botanist, from the important castor-growing tracts of the Central Provinces, India. The classification work, with a view to find out strains with high oil contents and yield, was started by Mr. H. P. Dwivedi. From this culture several true-breeding types with respect to certain characters have been established. Material for the present investigation was obtained from these types.

II. Material and technique

The following formula was used in fixing the meiotic chromosomes in smears only :—

One per cent chromic acid	5 c.c.
15-20 per cent formalin	2 c.c.
Glacial acetic acid	1 c.c.

This was followed by the usual iodine-gentian-violet method of staining. The material was fixed from two isolated types of which one was of the constitution **mGeBCLS** while the other one was not recorded.

III. MEIOSIS

This process was studied mainly from diakinesis onward. At diakinesis only one cell was observed which showed ten bivalents. No nucleolus was detected. Pro-metaphase was clearly observed. At I metaphase primary pairing is regular and ten bivalents are regularly formed. Secondary associations were studied at I and II metaphase, the maximum number being five. Six plates are drawn (Figs. 1-6) to illustrate various types of associations observed. From the plant which was not recorded, only three II metaphase plates were observed which showed the following results.

Groups	Number of secondary associations
1. 3(1)+2(2)+1(3)	4
2. 7(1)+1(3)	2
3. 4(1)+3(2)	3

From the other type of the constitution **mGeBCLS** two plants were examined at I and II metaphase and the results are shown in Table I. Twenty-eight plates were observed as shown below :—

	I metaphase	II metaphase
Plant I	11	2
Plant II	2	13
	13	15

TABLE I

Secondary associations at I and II metaphase in Ricinus communis, *2n=20*

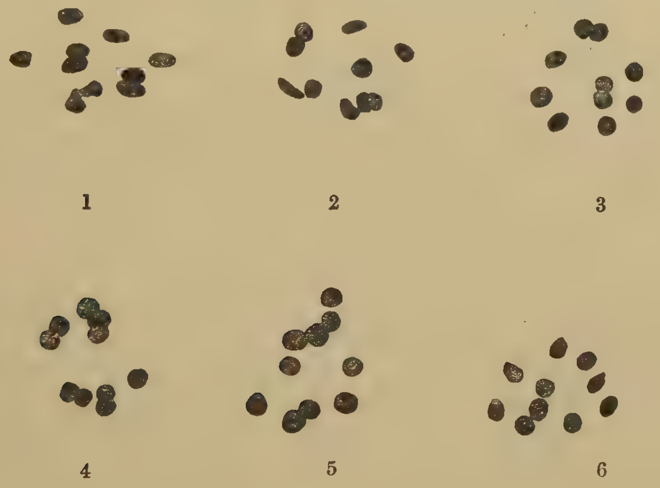
Number of secondary associations	Bivalents in groups of			Frequency of pollen mother cells	Total
	1	2	3		
0	..	..	..	..	..
1	8	1	..	3	3
2	6	2	..	5	6
	7	..	1	1	
3	4	3	..	6	12
	5	1	1	6	
4	3	2	1	4	5
	2	4	..	1	
5	1	3	1	2	2
Total		..		..	28

Thus *Ricinus communis* appears to be a secondarily balanced polyploid and on the maximum number of associations observed its constitution may be expressed as shown below, but this should be regarded as provisional till more evidence from genetical data and somatic chromosomes is obtained.

Constitution of gametic set of Ricinus communis *L.*

A A A
B B
C C
D D
E

The disjunction of the constituents of bivalents at the first division and the separation of chromatids at the second division are quite regular.



FIGS. 1-6. II metaphase polar views showing various secondary associations in *Ricinus communis*. a=Number of associations (× about 3500).

FIG. 1. 4(1)+3(2) ; $a=3$.

FIG. 2. 3(1)+2(2)+1(3) ; $a=4$.

FIG. 3. 6(1)+2(2) ; $a=2$.

FIG. 4. 1(1)+3(2)+1(3) ; $a=5$.

FIG. 5. 5(1)+1(2)+1(3) ; $a=3$.

FIG. 6. 7(1)+1(3) ; $a=2$.

IV. GENETICAL DATA FROM LITERATURE

Genetically the species has been investigated by White [1918], Harland [1920] and Peat [1928]. The latter has confirmed the results obtained by others and has analysed a few more factors. These results may be briefly mentioned as follows :—

1. The colour of the vegetative parts depends on the presence or absence of three independent factors, **MGE.**
2. The various types of leaf bloom depend on three separate factors, **BCD.**
3. Factors **P** and **A** control the colour of the seed-coat.
4. Factor **W** is responsible for the pattern of the seed-coat (*i.e.*, fine-dotted and spotted).
5. Further analysis of the fine-dotted class into ' simple fine-dotted ' and ' blotched fine-dotted ', possibly suggests two complementary factors controlling the two types. But as the author remarks that " at present this cannot be regarded as more than a suggestion." [Peat 1928].
6. Leaf-laciniation (factor **L**), spines (factor **S**) and albinism are individually controlled by single-factor pairs.

Factors **M** and **B** are linked with a cross-over percentage of about eight. No linkage relation was observed between any of the pairs studied by Peat in six factors, *viz.* **CPASGL.**

V. GENERAL CONSIDERATION

The data presented in this paper on secondary associations are in close agreement with the genetical results obtained by others. On the theory of secondary pairing [Darlington and Moffett, 1930 ; Lawrence, 1931 ; Catcheside, 1934], the associated chromosomes are supposed to be homologous. (This does not necessarily mean homology in all parts). The maximum number of associations observed is five, hence the gametic constitution of *Ricinus communis* may be expressed as **AAA BB CC DD E** with five as primary basic number (Fig. 4). Thus the castor-oil plant appears to be a secondarily balanced polyploid. This demands that in the genetical analysis of the species we would expect all types of segregations, *i.e.* mono-hybrid, di-hybrid and tri-hybrid ratio. The genetical literature on this species reviewed above, though not complete, clearly supports this demand.

VI. SUMMARY

The paper deals with the meiosis of *Ricinus communis* L. ($2n=20$) and a correlation between genetical segregation and secondary associations of chromosomes.

At diakinesis ten bivalents are formed. No nucleolus is observed.

At first metaphase primary pairing is regular. Secondary associations are very common and are investigated at first and second metaphase, the maximum number being five. Thus *Ricinus communis* appears to be a secondarily balanced polyploid and the constitution of its gametic set may be expressed as **AAA BB CC DD E**. This is supported by the presence of duplicate and triplicate factors in the genetical analysis of the species.

I am very grateful to Dr. W. Burns, D.Sc., I.A.S., for making valuable suggestions in preparing the manuscript. To Mr. J. C. McDougall, M.A., B.Sc., I.A.S., Director of Agriculture and Mr. K. P. Shrivastava, Second Economic Botanist, C. P., I am very much indebted for giving me all facilities to work in this Institute.

REFERENCES

Catcheside, D. G. (1934). *Ann. Bot.* **48**.

Darlington, C. D. and Moffett, A. A. (1930). *J. Genet.* **22**.

Harland, S. C. (1920). *J. Genet.* **10**, 207-218 ; **12**, 251-253.

Lawrence, W. J. C. (1931). *Cytologia* **2**.

Peat, J. E. (1928). *J. Genet.* **19**, 373-389.

White, O. E. (1918). *J. Hered.* **9**, No. 5.

OBSERVATIONS ON THE MILLING OF RICE IN THE UNITED PROVINCES

BY

R. L. SETHI, M.Sc., B.Sc.(Edin.), I.A.S.
Assistant Agricultural Expert, Imperial Council of Agricultural Research, India
B. L. SETHI, M.Sc., Ph.D.(Wales)
Officiating Economic Botanist (Sugarcane and Paddy) to Government, United Provinces
T. R. MEHTA, B.Sc.
Officiating Assistant Paddy Specialist
AND
P. S. GUPTA, M.Sc.(Luck.), M.Sc.(Lond.)
Research Assistant, Rice Research Station, Nagina

(Received for publication on 26th October 1936)

(With Plates LXII and LXIII and two text-figures)

CONTENTS

		Page
I.	Introduction	713
II.	*Sela*, parboiled and *kachcha* rices	714
III.	*Sela* and *kachcha* hulling	717
IV.	Hulling properties of different types	723
V.	Comparison of the efficiency of different appliances of hulling	724
VI.	Comparison of transplanted and broadcast paddy	728
VII.	Discussion	729
VIII.	Summary	731
	References	732

I. Introduction

Paddy is almost exclusively used for human consumption in the form of rice. It is clear that a study of the processes to which paddy is subjected before it is sold as rice in the market is important. Unfortunately most of the research done on paddy in recent years has been confined to the improvement of quality and yield of paddy by hybridization, selection, better methods of cultivation and manuring, and very little systematic study has been made of the milling of rice (*i.e.*, production of rice from paddy). To-day we are hardly in a position to judge how far a particular practice is the outcome of any kind of experiment or of blind tradition, or indeed to gauge how far the merits of one practice exceed those of another. General beliefs on the matter there have been and will possibly always be, but these same beliefs often come into conflict, the adherents of one practice denying the merits of a different practice. This points out the need for research.

Milling removes the hull, the bran layers, and the germ, leaving only the starchy endosperm (*i.e.*, the rice grain as we see it in the market). This treatment gives the rice the bright colour and pleasing appearance demanded by the consumer. The market value of the rice depends upon its appearance, and the fragrance and palatability of the food cooked from it.

All the rice-growing tracts practise milling, not only because it is an essential preliminary process of converting the commodity into a consumable form, but also because it results in economy in the rice trade by reducing the commodity by about fifty per cent in bulk and by about twenty-five per cent in weight, thus reducing the transport charges. Milling is an essential concomitant of rice cultivation, being practised either as a village industry or on a greater commercial scale, according to the extent of the trade of the milled product within a locality or between different localities.

Since the first process in milling is the removal of husk or hull (*i.e.*, hulling) and since hulling is accomplished by subjecting paddy to a mechanical force, a certain proportion of the rice or kernels is broken as a result. Broken rice sells for less and is graded lower than whole rice. Hence that variety which breaks less or that process which causes less breakage in a particular variety is to be preferred. It is easy to imagine how the diversity of taste of different peoples as well as the peculiarities of local conditions and local varieties, have resulted in several different milling practices and appliances coming into existence in different parts of the world. The United Provinces have not been an exception. The attempt of the present article will be to discuss some of these and to describe the result of experimental work done in this connection at the Rice Research Station, Nagina, during the last three years.

II. *SELA*, PARBOILED AND '*KACHCHA*' RICES

Parboiling of paddy, a treatment preliminary to milling proper, is a general practice in the United Provinces in common with other provinces of India and many other parts of the world, such as Burma, Ceylon, Malaya and British Guiana. Besides the usual method of parboiling, another method of treating paddy is followed in the western United Provinces, chiefly in the Saharanpur District. It is known as *sela*-making, the treated milled rice being called '*sela*' to distinguish it from the rice milled raw (*i.e.*, *kachcha* rice). Although essentially a method of parboiling, *sela*-making may be treated as a method by itself because of its fundamental difference from the method of parboiling usually practised. The product obtained by this method, *i.e.* the *sela* rice, is superior in certain important respects to parboiled and *kachcha* rices. That this is so, is substantiated by the fact that *sela* rice is in demand in Burma, Bengal, Bihar and Orissa, although these provinces mill large quantities of parboiled rices of their own.

We may shortly describe the *sela*-making process here.

Just as parboiled paddy has been termed steam-boiled or water-boiled paddy, *sela* paddy has been called lightly roasted paddy, although the word roasted is likely to over-emphasize the idea of gently heating the paddy, for that is what is actually done. The heating is done by means of hot sand. To begin with paddy is first steeped in fresh water in tanks for twenty-four to forty-eight hours. It is then taken out and gently roasted in hot sand contained in an iron pan heated by means of a *bhatti* (small furnace) fed by paddy husk. The paddy is briskly stirred along with the sand by means of an iron ladle provided with a wooden handle (Fig. 1). Experience is needed for judging the correct temperature of the sand and the correct duration for which the paddy should be allowed to remain in the sand. The heat should be just enough to evaporate the moisture from the wet paddy, instead of parching the rice. Charges of about eight pounds of paddy heated in a pan of about three feet diameter require four to five minutes to expel the moisture from the paddy. The paddy is then further dried in the sun for two days and then milled in power hullers. It is believed that besides imparting to the rice all the other advantages which are associated with parboiling, *viz.* increased resistance to breakage in milling, better nutritive properties and greater power of retention of shape and palatability on cooking, *sela*-making imparts a pleasing fragrance to the rice which is not obtained by parboiling.

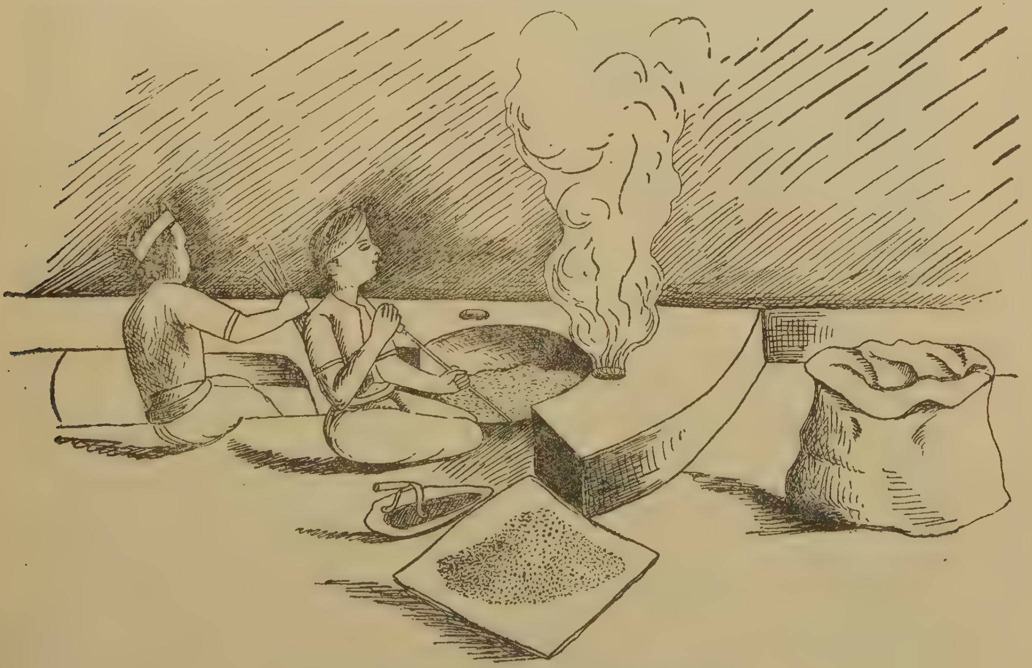


FIG. 1. *Sela*-making furnace in operation.

In parboiling the soaked paddy (sometimes foul-smelling as a result of repeated use of the same water for steeping for long periods) is heated by means of steam, or the steep-water containing the paddy is boiled for a short time. The millers in the Saharanpur District believe that rice thus boiled does not possess as great water-absorbing powers as does the *sela* rice, because the rice virtually cooked once has been deprived of some of its water-absorbing capacity, nor does it retain all the water it has absorbed during cooking when allowed to stand for an hour or two after cooking. In spite of parboiling being a method better adapted to handling large quantities of paddy more cheaply and in less time than is the case in *sela*-making the Saharanpur millers eschew the former method on account of their belief in the superiority of the *sela* rice.

The milling of *kachcha* (raw) rice is also widely prevalent in the United Provinces, both in the eastern as well as the western districts. Even in Saharanpur the milling of *kachcha* rice is an important industry, such rice being in greater demand by the richer classes of the provinces, the *sela* rice being in greater demand outside the provinces. Equalling the *sela* rice in quality, and sometimes surpassing it, are the *kachcha* rices of Dehra Dun, which are famous all over India as high quality rices. These include the famous *bansmati* and *hansraj* varieties, which are long and slender-grained, have an attractive appearance, and which elongate remarkably on cooking, giving a product which some consumers greatly relish. Rice millers at Dehra Dun produce only *kachcha* rice, although they handle rices which are slender and long and therefore liable to much breakage in milling. They do not appear to attach significance to the general belief that parboiling or *sela*-making reduces breakage in milling, especially when power-hullers of the Engelberg type are used. Such a belief is expressed by Douglas [1930] when, while discussing rice milling in British Guiana, he states "The present method of milling by means of what is known as the Engelberg Huller is undoubtedly effective up to a point when treating parboiled rice. It is, however, unsuited to milling anything but parboiled, and therefore toughened and easily shelled, grain. Moreover the loss of husk, valuable bran and broken rice is considerable."

Clearly, therefore, there was a necessity of subjecting the two methods of hulling paddy, *viz.* the *sela* and the *kachcha*, to comparative tests in order to discover how one method compared with the other and what factors contributed to the industrial success of the two rival methods practised in adjoining districts. That such a work would be of economic significance may be taken for granted, because it is only the Dehra Dun, the Pilibhit and the Saharanpur rices (both *sela* as well as *kachcha*) which find a market outside the provinces. Every year about half a million maunds (one maund=eighty-two pounds) of such rice are exported to other Indian provinces. The rice sent from Delhi to Surat, Bombay, Gujrat, etc., and known as Delhi rice is really the Dehra Dun or the Saharanpur rice. As a consequence of the attempt of the Department of Agriculture of the United

Provinces to distribute long-grained, good quality rices among the cultivators the possibilities of expanding the export trade of these rices have increased. A thorough study of the processes of milling followed in Dehra Dun and Saharanpur is, therefore, likely to yield information which may guide future practices and effect economies in various directions.

III. *SELA* AND *KACHCHA* HULLING

In the winter of 1933 a programme of milling tests was laid down to investigate, among other questions, the comparative efficiency of the *sela* and the *kachcha* methods of hulling employing power hullers. '*Sela*-making' is a process which requires experience and care, hence the services of an expert person from Saharanpur had to be requisitioned to carry on the trials at Nagina. Large paddy tanks and steeping of large quantities of paddy being out of question for preparing *sela* paddy for experimental purposes, the processes were carried out on a smaller scale. The details are given below.

Paddy in suitable lots (about 30 lbs.) was immersed in fresh water in wide-mouthed earthenware vessels for about thirty-six hours, after which it was stirred with the hands. Then the excess of water containing the husk, light grains, straw, etc. was drained off, and the soaked paddy transferred to bamboo baskets lined with gunny bags in order to drain off more water. This soaked paddy was put (in six to eight-pound lots) in the hot sand contained in an iron pan placed over the furnace fed with paddy husk. All the time the paddy was in the pan it was thoroughly stirred by means of a ladle (Fig. 1). The temperature of the sand was not ascertained, but it may be said to have been near 'red heat'. The time of frying ranged between three and five minutes for each lot of paddy. The heated paddy was then separated off from the sand by sifting the mixture of sand and paddy by means of a sieve which allowed the sand to fall back into the pan but not the paddy. This was followed by a fresh charge of paddy into the sand. The 'roasted' paddy was kept overnight in gunny bags so as not to lose its temperature rapidly. Next morning the paddy was spread in a thin layer on a *pucca* (brick) floor and exposed to the sun and stirred periodically to help uniform drying. The drying was continued for two or three days according to the conditions demanded by the experiment or the weather. Then the paddy was hulled.

In *kachcha* hulling the paddy as obtained from the storage bags or bins is not given any preliminary soaking or heating treatment but only exposed to the sun for two or three days prior to milling.

The milling of the two kinds of paddy was done by means of the Engelberg huller (a power huller manufactured by the Engelberg Huller Company of Syracuse, New York, U. S. A.). This is a very efficient machine and is especially suitable for small mills. The milled rice was sifted from the husk and admixtures by being tossed deftly in a *soop* (a flat straw hand winnower with edges raised on three sides and worked usually by a woman who holds it at the two ends with her hands). The cleaned rice (containing whole and broken grains) was placed in a grain mixer and the rice mixed thoroughly. Samples from these were taken out and passed through Cole's sample separator [Hayes, 1932] in order to separate the whole grains from the broken. The percentages of whole grains on the paddy hulled were worked out. The trials were carried on in 1933, 1934 and 1935, with different types including early, medium-early and late rices. In each type the milling as well as the preliminary treatments were repeated four times. Thus in each year a set of four percentages was obtained for each method of hulling in each type. The percentage figures were subjected to analysis of variance and the significance of differences measured.

The types used were T 1, T 3, T 7, T 12, T 21, T 23, T 26 and T 100. T 1, T 7, and T 12 are early types, T 3 and T 21 are medium-early and the last three are late in maturity. The grains of T 1, T 3 and T 7 are long and narrow, of T 12, T 21 and T 23 long but bolder than those of T 1 or T 7, of T 26 medium and narrow, of T 100 coarse. The measurements of grain (paddy) are given in Table I.

TABLE I

Grain measurements of Types 1, 3, 7, 12, 21, 23, 26 and 100
(Averages of twenty measurements)

Type	Grain length in mm.	Grain width in mm.	Ratio (length : width)
T 1	10·48 ± 0·35	2·23 ± 0·08	4·72 ± 0·93
T 3	9·93 ± 0·27	2·13 ± 0·07	4·67 ± 0·02
T 7	8·78 ± 0·51	2·08 ± 0·07	4·20 ± 0·02
T 12	9·93 ± 0·48	2·52 ± 0·16	3·96 ± 0·30
T 21	10·38 ± 0·42	2·70 ± 0·07	3·85 ± 0·01
T 23	9·54 ± 0·35	2·33 ± 0·14	4·11 ± 0·31
T 26	7·81 ± 0·31	2·09 ± 0·06	3·73 ± 0·02
T 100	7·97 ± 0·27	3·60 ± 0·16	2·21 ± 0·01

To minimise space the data and the details of analysis will not be presented here. The summaries of results for the three years are given in Table II.

Table II

Comparison of sela *and* kachcha *methods and the efficiency of different types as regards their hulling qualities in different years*

Varieties	Percentages of whole grains			Percentages of mean		
	Sela	*Kachcha*	Mean	*Sela*	*Kachcha*	Mean
			(1933)			
T 1	53·7	53·6	53·6	94·4	94·2	94·3
T 7	67·7	45·4	56·5	119·0	79·8	99·3
T 12	62·8	52·8	57·8	110·4	92·8	101·6
T 21	36·1	52·7	44·4	63·4	92·6	78·0
T 26	70·0	60·4	65·2	123·0	106·2	114·6
T 100	64·7	63·2	63·9	113·7	111·1	112·3
Mean	59·2	54·7	56·9	104·0	96·1	100

S. E. for single treatment	3·25	or	5·71	per cent
S. E. for varieties	2·30	or	4·04	,,
S. E. for methods of hulling	1·33	or	2·33	,,
Critical difference for method of hulling when each variety is considered separately	9·39	or	16·49	,,
Critical difference for method of hulling when all varieties are considered	3·84	or	6·73	,,

Varieties	Percentages of whole grains			Percentages of mean		
	Sela	*Kachcha*	Mean	*Sela*	*Kachcha*	Mean
			(1934)			
T 1	59·4	52·1	55·7	116·4	102·1	109·2
T 3	45·8	41·6	43·7	89·7	81·5	85·6
T 7	57·7	34·9	46·3	113·0	68·4	90·7
T 12	54·2	50·0	52·1	106·2	97·9	102·0
T 21	53·3	48·8	51·1	104·4	95·6	100·0
T 23	63·9	49·3	56·6	125·2	96·6	110·9
T 100	59·1	44·6	51·8	115·8	87·4	101·6
Mean	56·2	45·9	51·05	110·1	89·9	100

S. E. for single treatment	1·55	or	3·04	per cent
S. E. for varieties	1·10	or	2·15	,,
S. E. for methods of hulling	0·59	or	1·15	,,
Critical difference for method of hulling when each variety is considered separately	4·48	or	8·78	,,
Critical difference for method of hulling when all varieties are considered	1·70	or	3·32	,,

Varieties	Percentages of whole grains			Percentages of mean		
	Sela	*Kachcha*	Mean	*Sela*	*Kachcha*	Mean
			(1935)			
T 1	64·1	57·8	60·9	103·6	93·4	98·5
T 3	54·7	58·6	56·6	88·4	94·7	91·5
T 12	68·4	52·7	60·5	110·5	85·1	97·8
T 21	53·3	56·0	54·6	86·1	90·5	88·3
T 23	68·8	67·5	68·1	111·1	109·0	110·0
T 100	72·7	68·4	70·5	117·4	110·5	113·9
Mean	63·7	60·2	61·9	102·8	97·2	100

S. E. for single treatment . . . . 2·63 or 4·25 per cent

S. E. for varieties . . . . . 1·86 or 3·01 „

S. E. for methods of hulling . . . . 1·07 or 1·74 „

Critical difference for method of hulling when each variety is considered separately . 7·59 or 12·27 „

Critical difference for method of hulling when all varieties are considered . . . 3·09 or 5·02 „

The data given in Table II are illustrated in Fig. 2. It will be noticed, both from the table as well as from the figure, that the differences in percentages of whole grains derived from *sela* and *kachcha* paddies vary in magnitude from variety to variety and from year to year. Comparing the two methods variety by variety with the help of the figures for critical difference given in the table we find that the percentages given by *sela* paddy are significantly higher than those given by *kachcha* paddy in about half the comparisons. In the majority of the remaining comparisons the figures given by *sela* paddy, though higher, fail to satisfy statistical demands to prove their superiority. Only in one case is the figure for *sela* statistically inferior to that of *kachcha*. When, however, we consider all the types together the *sela* process has given higher percentages of whole rice than the *kachcha* process by the following amounts in different years.

1933 . . . . 7·9 per cent

1934 . . . . 20·2 „ „

1935 . . . . 5·6 „ „

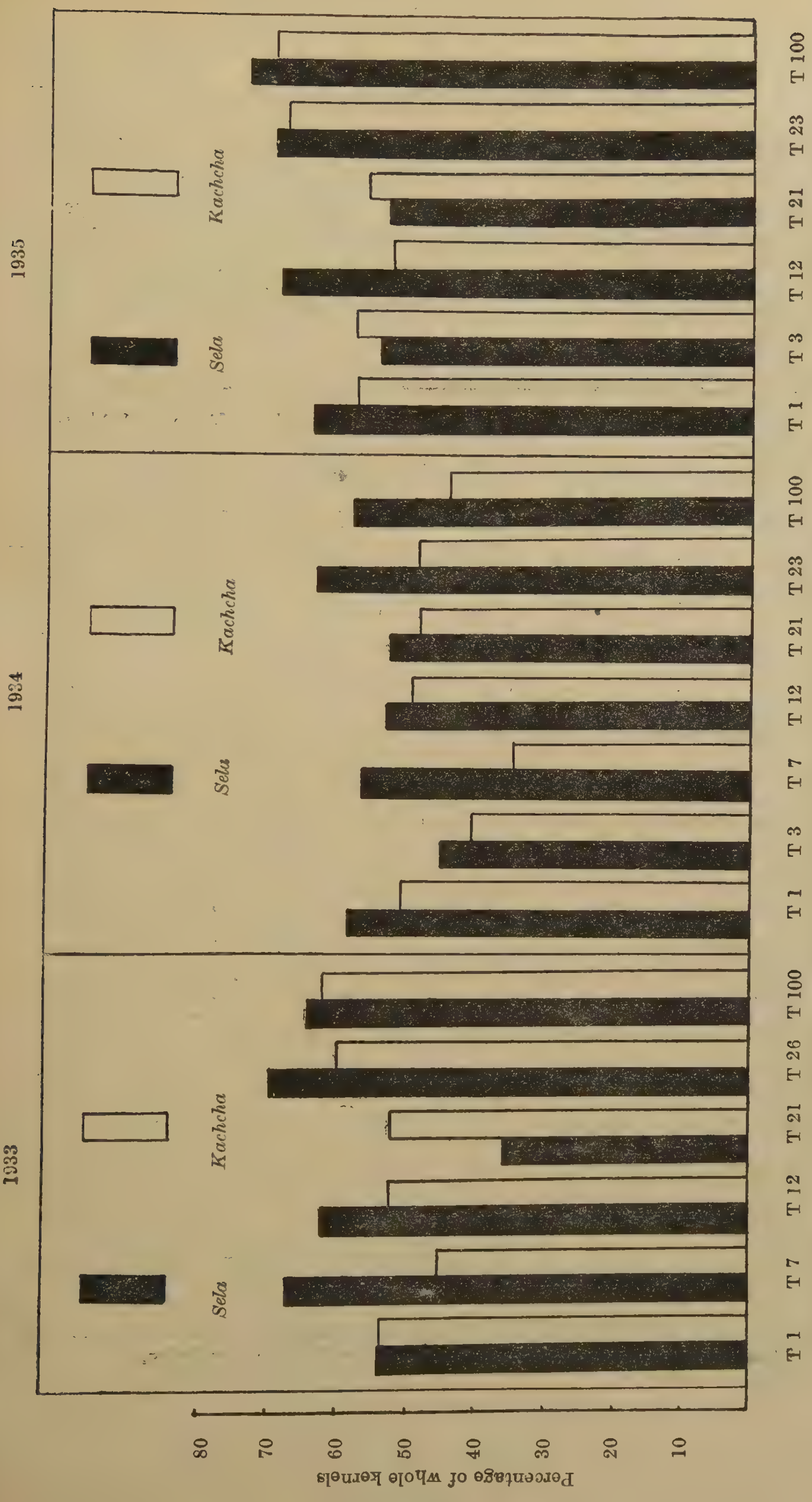


FIG. 2. Percentage of whole grains obtained by *sela* and *kachcha* method of hulling in different types in 1933, 1934 and 1935

The critical differences for the three years are, respectively, 6·73, 3·38 and 5·02 per cent, thus showing that the above differences are significant.

That the percentages of whole grains vary from variety to variety and from year to year is made more apparent by the study of variances given by the different variables that enter into the experiment. Consolidating the three years' data gives us the following analysis of variance in which the different interactions are also presented :—

Analysis of variance

Due to	Degrees of freedom	Sums of squares	Mean squares	1/2 loge
Replications	3	128·15	42·72	1·8773
Varieties	3	1801·01	600·34*	3·1987
Seasons	2	1646·13	823·07*	3·3565
Methods	1	312·13	312·13*	2·8717
Interactions				
Replications × Varieties . . .	9	128·50	14·28	1·3294
Replications × Seasons . . .	6	208·58	34·76	1·7743
Replications × Methods . . .	3	49·29	16·43	1·3995
Varieties × Seasons	6	956·86	159·48*	2·5359
Varieties × Methods	3	615·04	205·01*	2·6616
Seasons × Methods	2	326·98	163·49*	2·5484
Replications × Varieties × Seasons .	18	492·78	27·38	1·6549
Replications × Varieties × Methods .	9	405·94	45·10	1·9045
Replications × Seasons × Methods .	6	264·72	44·12	1·8934
Varieties × Seasons × Methods . .	6	429·28	71·55	2·1352
Error . .	18	707·82	39·32	1·8358
Total .	95	8473·21		

*** The variances marked by asterisk are of significant magnitudes.**

IV. Hulling properties of different types

The above trials also aimed at yielding information on the hulling properties of different types. Since the experiments were planned systematically the data used for comparing the *sela* and *kachcha* hulling can serve the dual purpose of comparison of the susceptibility of the different types to breakage in hulling. The types used are among those which have been distributed from the Department as improved strains. Besides, they represent a diversity of sizes and shapes of grains. The selection of these diverse types for the milling trials was done on purpose. It is apparent that such a procedure would yield information on the behaviour of widely different kinds of rices in milling.

The summaries of results of the three years have already been presented (Table II). The milling tests were conducted in the months of January, February and March in each year with the paddy harvested in the preceding *kharif* (autumn) season. The early varieties were harvested in October and the late varieties in November and early December. Thus when the tests started, the two kinds of paddies had enjoyed different periods of storage. The analysis of the results showed that in general the late types, *viz.* T 23, T 26 and T 100 gave higher percentages of whole grain as compared to the early types. This difference was not due to the difference in the periods of storage enjoyed by the two groups of paddies, because the examination of the effect of storage (conducted in forty types in 1935) shows that the storage tends to improve the percentages rather than decrease them. Hence we are inclined to attribute the differences in hulling in the two cases to difference in the seasonal factors existing at the time the respective crops matured. Moreover, the post-harvest operations, such as threshing, drying, etc., being conducted under very different conditions may be largely responsible in affecting the milling quality of a type. That such can be the case may be concluded from the observations of different workers on paddy. Thus, for example, Stahel [1935] and Smith [1928] have found that the breakage in milling is partly dependent upon the amount of moisture present in the paddy at the time of milling, which is dependent upon the amount of drying given to the paddy and upon the conditions of storage. Further Stahel [1935] has shown that the age of the paddy at harvest exerts profound influence on percentage of whole rice, the percentage increasing as the rice ripens, but again showing a decline after the harvesting period has reached a certain stage. This rise and decline was found to be related to the moisture content below or above a certain critical value resulting in increased breakage. Further Stahel found that remoistening paddy which has been dried below a certain critical moisture-content level causes the development of 'sun-cracks' and a consequent increase in breakage in milling. He recommends that stored paddy should not be allowed to dry out beyond this level. It is apparent that if the existence of such a critical level is true of every variety, soaking of paddy (as is done in parboiling or *sela*-making) which has been dried beyond the critical level is bound to result in increased breakage in milling. The method of drying has also been found to affect the milling properties.

In view of these facts it is easy to understand why a particular type hulled in different years can yield different percentages of whole rice. This can be clearly seen in our experiments by a glance at Table II. Thus we may state here that although varietal differences are important in milling, the environmental factors, some of which are out of our control, may be of even greater importance in determining the milling qualities of rice.

V. COMPARISON OF THE EFFICIENCY OF DIFFERENT APPLIANCES OF HULLING

In a great part of the United Provinces milling of rice is done by the primitive method of simple pounding, using hand power or foot power. It was decided therefore to test the efficiency of this method against that of power hullers. Incidentally, it was thought desirable to test the efficiency of two experimental hulling appliances, one a small hand-huller known as 'Chotadost' (Plate LXII, fig. 1) and the other a three-pestle pounder called 'Moung' (Plate LXIII, fig. 1) and used in Burma for testing paddy [Hayes, 1932]. Brief descriptions of the four appliances used in the trials may be given here.

Engelberg huller.—As already stated it is a power huller efficient for small mills. In the western districts of the United Provinces most of the millers use this huller, the electric power being cheap. The paddy is fed into the machine through the receptacle at the top whence it passes over an iron roller provided with longitudinal grooves. The paddy is crushed between the surface of the roller, an iron plate running parallel to it as the former revolves. The space between the roller and the plate is adjustable by means of screws, thus allowing the machine to hull efficiently grain of different sizes. The hulled grain and the powdered husk then pass into a screen where the two are separated. The hulled grain emerges through a sprout in front while the husk, small bits of kernel and rice polish are carried below the machine and finally away from it through another outlet. The huller is capable of hulling about 600 pounds of paddy in one hour.

'*Chotadost*' *hand huller.*—As the name implies it is a small hand machine working on the same general principle as the Engelberg huller. It is eminently suited for hulling small samples of paddy for experimental purposes.

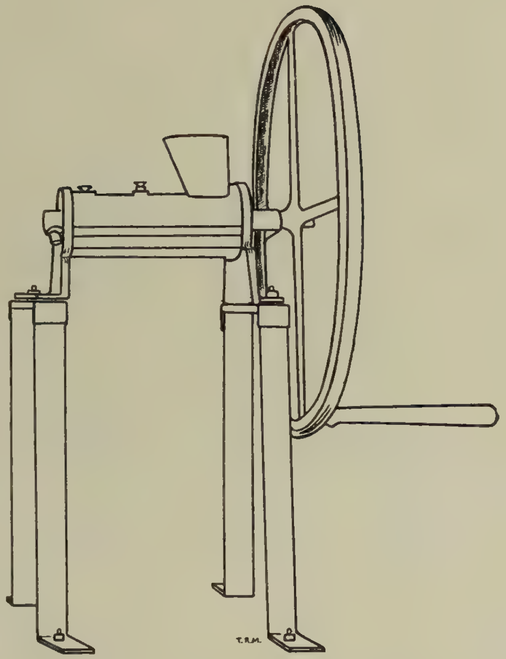


FIG. 1. 'Chota dost' hand huller

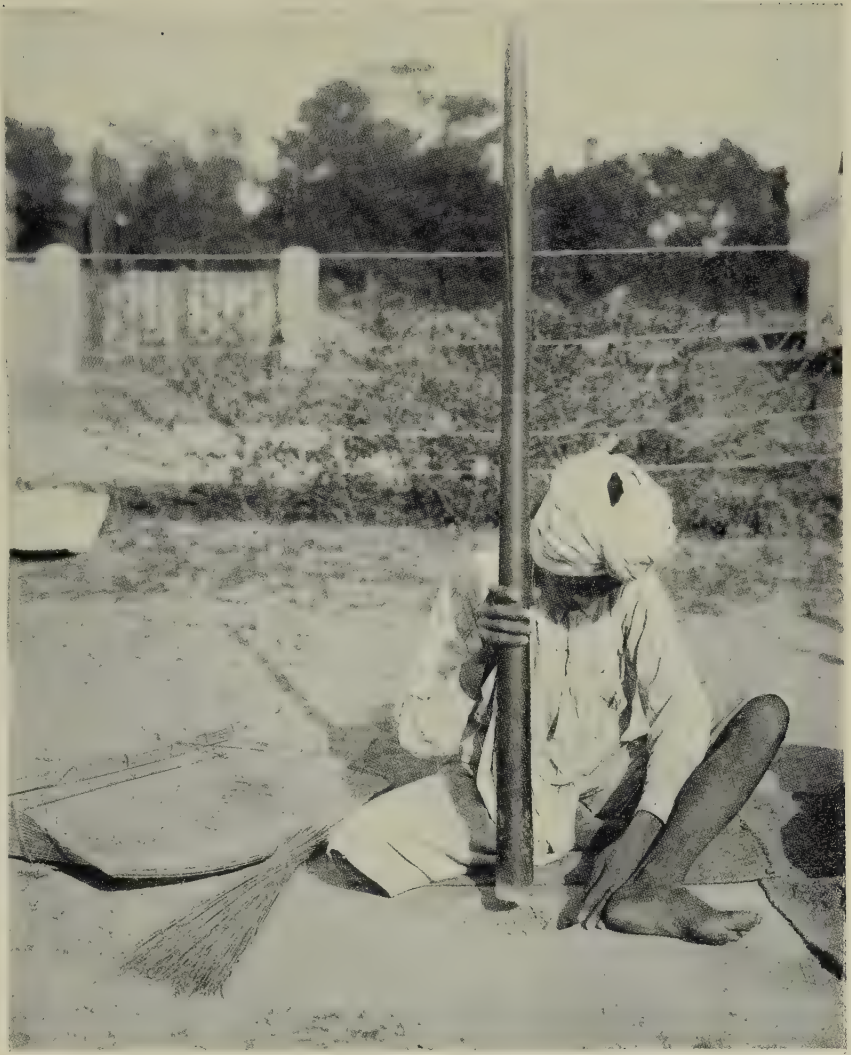


FIG. 2. The country method of hulling

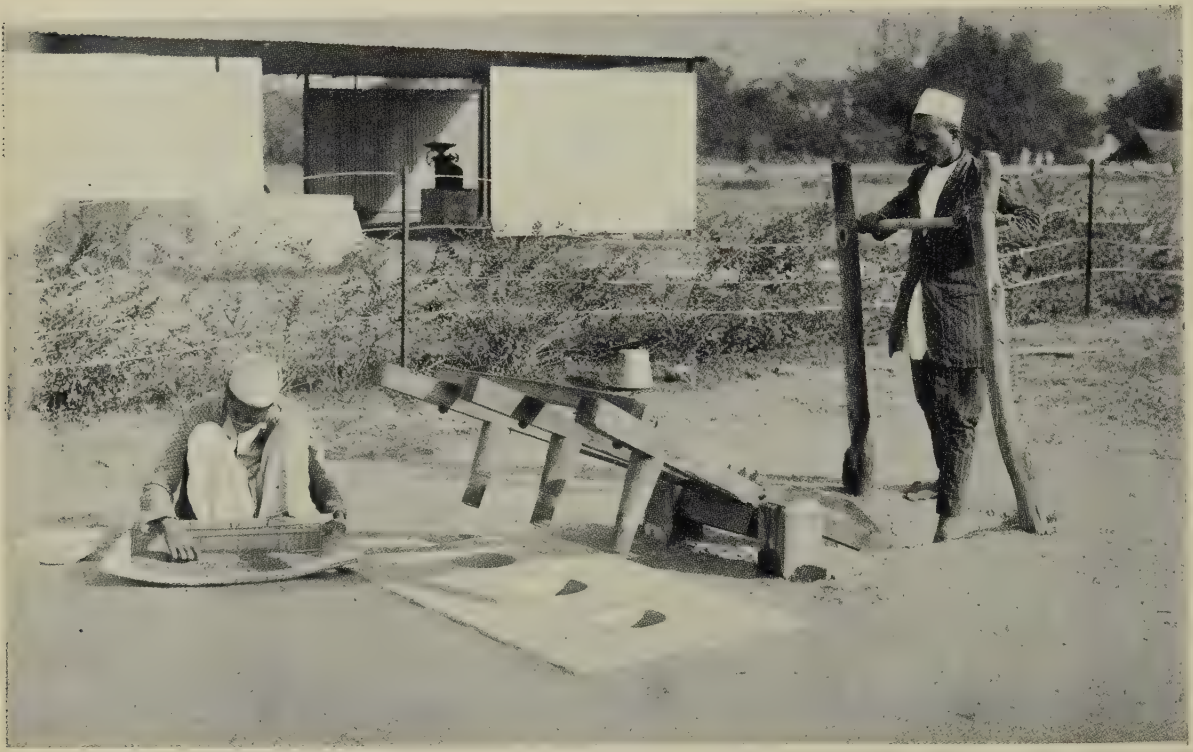


Fig. 1. Moung (Grain pounder)

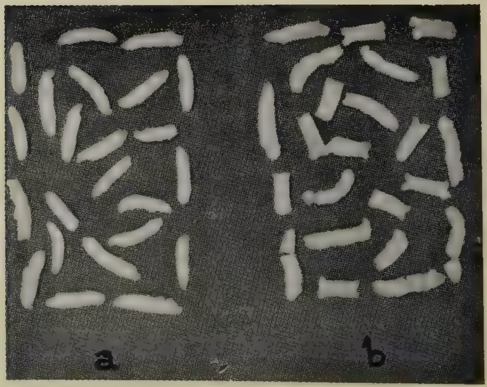


Fig. 2. *Sela* (*a*) and *kachcha* (*b*) rices of T 3 cooked for twenty-five minutes. The former has kept the grain shape intact but not the latter.

Country method.—It consists of simple pounding. The method is crude and laborious. The principal implements used are a wooden mortar and a wooden pestle shod at the striking end by an iron ring. The pestle is either a light wooden rod four or five feet long and worked by hand or a heavy wooden lever about seven feet long, provided with a pestle at one end and worked by a man alternately pressing down and releasing the other end with his feet. For the trials described in this paper the hand pestle was used (Plate LXII, fig. 2). The size of each charge was about a pound or two, the pestle being worked by a man who pounded the paddy with one hand and kept it stirred in the mortar with the other.

Moung.—This appliance is a modification of the country pounder worked by the foot, the modification consisting of the fact that the central beam of the lever has two arms attached to it so that the striking end consists of three beams each bearing a pestle (Plate LXIII, fig. 1). Working the beams at the opposite end by means of the foot brings into action all the three pestles simultaneously, each in a separate mortar. This appliance enables the experimenter to use one of the mortars for holding a 'control' sample and the other two for holding samples which are to be compared with the control. The moung has been described in detail by Hayes [1932].

The milling trials were carried out in 1933, 1934 and 1935. The first year trials were of a preliminary nature, and the data obtained are not considered suitable for presentation. The results of the trials in the two later years alone are presented here, but the conclusions of the three years are more or less in agreement. With every appliance the paddy used was either *kachcha* (raw) or pre-treated as *sela*. Further, the source of the paddy was either a transplant crop or a broadcast one. Thus the total number of treatments tested was sixteen *viz.* four hulling appliances × two methods of culture (transplant and broadcast) × two methods of hulling (*kachcha* or *sela*). Each treatment was repeated four times. The quantity of paddy hulled with each of the different methods of hulling was a convenient minimum arrived at during the course of the trials. In the case of the Engelberg huller each charge was fifteen pounds, and in the other methods it was two pounds in each case. The broken and whole kernels were separated as already described. The summary of the sixty-four hulling operations in each year is given in Table III.

Table III

Percentage of whole kernels on paddy hulled in T 1 grown under transplant and broadcast conditions and hulled raw or as sela *using different appliances*

Huller	Percentages of whole grains					Percentage of mean				
	Transplanted		Broadcast		Mean	Transplanted		Broadcast		Mean
	Sela	Raw	*Sela*	Raw		*Sela*	Raw	*Sela*	Raw	
					(1934)					
'Engelberg'	59·4	52·1	55·0	48·4	53·7	122·2	107·2	113·2	99·6	110·5
'Chotadost'	54·6	44·3	51·2	46·7	49·2	112·3	91·2	105·3	96·1	101·2
'Moung'	54·2	55·1	50·1	47·7	51·8	111·5	113·4	103·1	98·1	106·6
'Country method'	49·9	27·5	53·8	27·0	39·5	102·7	56·6	110·7	55·6	81·3
Mean	54·5	44·8	52·5	42·5	48·6	112·1	92·2	108·0	87·4	100

Standard error for 1 treatment	2·02 or 4·17 per cent
Standard error for mean of 4 treatments	1·01 or 2·09 ,,
Standard error for mean of 8 treatments	0·715 or 1·48 ,,
Critical difference for method of hulling (*i.e.*, *sela* or raw)	2·08 or 4·27 ,,
Critical difference for hulling appliance	2·92 or 6·04 ,,

TABLE III—*contd.*

Huller	Percentages of whole grains					Percentage of mean				
	Transplanted		Broadcast		Mean	Transplanted		Broadcast		Mean
	Sela	Raw	*Sela*	Raw		*Sela*	Raw	*Sela*	Raw	
					(1935)					
'Engelberg'	64·1	57·8	65·7	60·0	61·9	108·2	97·6	110·9	101·3	104·5
'Chotadost'	59·9	57·0	63·6	58·7	59·8	101·1	96·2	107·3	99·1	100·9
'Moung'	59·8	55·3	62·1	55·7	58·2	100·9	93·3	104·8	94·0	98·2
'Country method'	61·8	55·8	57·9	52·9	57·1	104·3	94·2	97·7	89·3	96·4
Mean	61·4	56·5	62·3	56·8	59·25	103·6	95·4	105·1	95·9	100

Standard error for 1 treatment	1·63 or 2·75 per cent
Standard error for mean of 4 treatments	0·81 or 1·37 ,,
Standard error for mean of 8 treatments	0·58 or 0·97 ,,
Critical difference for method of hulling (*i.e.*, *sela* or raw)	1·67 or 2·80 ,,
Critical difference for hulling appliance.	2·34 or 3·96 ,,

An examination of the percentage figures given in Table III in relation to the values of critical differences shows that in both the years the country method gave significantly lower percentages of unbroken kernels than the other appliances. The highest values were recorded by the Engelberg huller, which was significantly superior to Chotadost hand huller in 1934 and to the Moung in 1935, the latter two being about equal in efficiency.

VI. COMPARISON OF TRANSPLANTED AND BROADCAST PADDY

The above trials yield information on the question of the relative efficiency of transplanted and broadcast paddy for milling as well and also give some light as on seasonal variation. This information and also the interactions between the different factors (*e.g.*, season, cultural method, method of hulling and hulling appliance) have been secured by combining the two years' data and calculating the analysis of variance. The analysis is given below.

Analysis of variance

Due to	Degrees of freedom	Sums of squares	Variance	½ log
Appliance	3	383·67	127·89*	2·4256
Method	1	458·29	458·29*	3·0638
Culture	1	4·58	4·58	0·7609
Season	1	914·85	914·85*	3·4094
Appliance × Method	3	161·63	53·88*	1·9934
Appliance × Culture	3	11·27	3·76	0·6622
Appliance × Season	3	142·74	47·58*	1·9312
Method × Culture	1	0·38	0·38	..
Method × Season	1	44·42	44·42	1·8968
Culture × Season	1	15·54	15·54	1·3717
Appliance × Method × Culture	3	6·46	2·15	0·3827
Appliance × Method × Season	3	155·60	51·87*	1·9744
Method × Culture × Season	1	0·03	0·03	..
Error	6	56·97	9·49	1·1251
Total	31	2356·43		

*Variances marked with asterisks are significant as tested by Fisher's '*z*' at five per cent *P*. Thus it is seen that the difference in milling due to cultivation is not significant. Seasonal variation is considerable. The interactions between season and appliance, season and method, and season, appliance and method are significant.

VII. DISCUSSION

In the experiments described above the differences observed in the milling qualities of the different types of paddy were distinct but were not constant from year to year. These facts may mean that milling, besides being a varietal characteristic, is largely dependent upon the environmental factors to which the grain is subjected at maturity and after harvest. The influence of these factors has already been discussed in the body of the paper (pp. 723, 724) and is under investigation at Nagina.

Sela-making reduces the amount of breakage. The gain to the miller is at the expense of the extra labour involved in making the *sela*. In our experiments the increase in the percentage of whole grain was not large enough completely to account for the popularity of this method. It is relevant to suppose that advantages other than that of reduced breakage in milling are associated with *sela* rice. Cooking tests carried out by us give the following information :—

1. *Sela* rice is more delicious than *kachcha* rice on cooking.

2. Cooked *sela* rice retains its shape and flavour longer when allowed to stand for a number of hours.

3. The amount of time required for the *sela* rice to cook is longer.

4. Prolonged cooking improves the quality of the *sela* rice, enabling it to absorb more water without the grains losing their shape, while the grains of the *kachcha* rice, with the same amount of absorption, lose shape (Plate LXIII, fig. 2). Such rice may, as a result of bursting of most of the grains ultimately imbibe more water than the unburst *sela* rice and form a pasty mass. However, a thoroughly cooked sample of *sela* rice contains more water than a sample of *kachcha* rice possessing the same degree of soundness of grain.

The millers when claiming the superiority of *sela* rice always emphasize the cooking quality of this rice.

It appears that *sela* rice is possessed of all the advantages which we associate with parboiled rices. The belief that it is more sustaining than *kachcha* rice is deep-rooted. A similar belief prevails about parboiled rice in different countries. Thus Douglas [1930] says about parboiled rice that "it is undoubtedly more nourishing than the highly finished and artificially glazed and coated rices of commerce.... It is, after all, in universal use among the white or European population of the West Indies in general ; and not only so, it is actually preferred by them to the less nourishing and more expensively milled white rice". The superiority of the nourishing quality of parboiled rice has been confirmed by several workers. Aykroyd [1932] found that for the same degree of milling the parboiled rice contained more of the anti-neuritic vitamin B than the raw rice and opined that in soaking and heating the paddy some of the vitamin B and the phosphate contained in the pericarp and germ diffuse through the endosperm. Recently a progress report on the research of quality in rice has been published by the Imperial Council of Agricultural Research, India [Anonymous, 1936]. This report shows that " parboiled milled rice is richer in nitrogen and phosphorus than raw rice, milled to an equal extent, yet, with a high degree of milling, this increase tends to rapidly diminish until with twenty per cent polishing, parboiled rice is only about as rich as raw rice in these two constituents."

Yet the Dehra Dun millers continue to prefer milling their paddy raw. Even at Saharanpur raw paddy is milled and sold. The reason why *sela*-making is not universally adopted in these parts of the United Provinces in spite of the obvious superiority of *sela* rice is probably that some orthodox Hindus of the U. P. would consider soaked and roasted paddy as having been handled too much and not meeting their caste requirements. Hence *sela* rice is more in demand by Mohammedan consumers. Yet the orthodox Hindu of epicurean taste must satisfy his palate. He therefore goes in for the *kachcha* milled rices of Dehra Dun and Saharanpur, and thus the two industries are flourishing side by side, the *sela* rice catering for the taste of one class of the population and the *kachcha* rice satisfying the palate of the other class.

It is evident that the long, slender grains, not toughened by being subjected to the process of *sela* making, will suffer enormous breakage in milling. It can roughly be stated that an average rice miller at Dehra Dun expects out of one maund of paddy (one maund = forty seers = eighty-two pounds) only about sixteen seers of whole rice, the rest of the product consisting of eight seers of broken kernels (*kanki*) and sixteen seers of husk, bran and rice polish. Thus he secures from his paddy only forty per cent of whole rice, which is a very small figure. If this figure could be increased his trade would benefit thereby. It is known that the conditions of harvesting, drying and storing generally given to paddy in these provinces are not such as to yield ideal returns in milling. Any change in these operations which may improve the milling quality of the paddy will obviously be of great benefit to the miller. The study of these operations and their effect on milling is under way at Nagina, and it may result in improvement of rice milling in the provinces as a whole. Among the problems being investigated are the following :—

(1) Effect of age of rice at harvest on its milling quality,
(2) effect of moisture-content of paddy on breakage,
(3) relation between moisture per cent and ' sun-cracking', and
(4) effect of different methods of stacking and drying on breakage.

When we recall the fact that certain countries, such as Upper Burma, Japan, Java, Italy, Spain and the United States, which mill only raw rice, have carried rice-milling to an advanced stage and that they regularly turn out high percentages of whole grains from their mills even when they use long-grained paddy, it is easy to understand how much of their success is due to careful handling of grain after harvest and the use of suitable milling appliances. It would be legitimate to expect, therefore, that the long and slender-grained rices of the United Provinces, such as the *bansmati*, can be milled more economically if better drying and storage practices are followed. The question is being investigated at Nagina and it may lead to definite recommendations in course of time. The Engelberg huller may have to be replaced by other appliances. Douglas [1930], writing about milling of rice in British Guiana, says that the Engelberg huller is unsuited to milling raw rices, and that instead a disc-sheller is used, followed by a winnower to remove the husk. Such an appliance may prove superior to the Engelberg huller in the United Provinces for the *kachcha* method of milling. Unless this method is made more economic there is little chance of raw milled white rices finding a market in Europe or America where there is a demand only for such rices.

VIII. SUMMARY

1. A method of pre-treating rice before milling (*sela*-making method) has been described. It is akin to parboiling, but imparts better qualities to rice than is usual with parboiled rice.

2. Milling of *sela* and *kachcha* (raw) paddy of long and slender-grained rices is an important industry in the west United Provinces. A comparison of the *sela* and the *kachcha* methods of milling showed a lower percentage of breakage of rice in the former case.

3. Breakage in milling is a varietal characteristic, and is, besides, largely governed by drying and storing operations.

4. Hulling properties of different improved varieties of paddy were tested.

5. Different appliances of hulling, *viz.*, Engelberg huller, Chotadost hand huller, country method and the Moung were tested.

6. The milling qualities of a type grown under transplanted and broadcast conditions were identical.

7. Cooking tests showed that the *sela* rice when cooked surpasses the raw rice in flavour, in keeping quality and in absorption of water consistent with retention of grain shape.

The writers are deeply indebted to the Imperial Council of Agricultural Research, India, for a grant of funds for rice research which has enabled them to carry on the above work.

REFERENCES

Anonymous (1936). Progress report on the scheme of research on 'Quality in crops with special reference to rice' for the year ending 30th November, 1935. Government of India Press, Simla, 1936.

Aykroyd, W. R. (1932). *J. Hygiene* **32**, 184.

Douglas, C. E. (1930). *Empire Marketing Board* **32**, p. 63.

Fisher, R. A. (1932). 'Statistical Methods for Research Workers', 4th Ed. Oliver and Boyd, London.

Hayes, A. (1932). *Agric. and Livestock Ind.* **2**, 162-169.

Smith, W. D. (1928). *U. S. Dept. Agric. Circ.* **8**.

Stahel, G. (1935). *Trop. Agric.* **12**, 255-261.

A NOTE ON A FEW EXPERIMENTAL OBSERVATIONS IN THE RICE RESEARCH STATION, BERHAMPUR (MADRAS)

BY

M. B. V. NARASINGA RAO, B.A., B.Sc. (Ag.), Assoc. I. A. R. I.

Assistant to Paddy Specialist, Coimbatore

(Received for publication on 15th March 1937)

CONTENTS

PAGE

1.—Experimental plot-size 733
  I. Introduction 733
  II. Material and methods 734
  III. Summary 737
2.—Correlation of some characters on yield in rice . . . 738
  I. Review of previous work 744
  II. Summary 744
  III. Acknowledgments 744
References 744

1. Experimental plot-size

I. Introduction

The optimum size and shape for an experimental plot is shown to vary with the crop under study and also with the particular locality. Parnell [1919] determined the soil variability in typical paddy soils of the Madras Presidency and found that the probable error of difference between two plots varied from 7·7 to 13·9 per cent. In Coimbatore, Ramiah [1931-32] concluded from uniformity trials in paddy that very small plots adequately replicated bring down the experimental error to any required precision. From the preliminary experiments conducted at Maruteru [1926] it was found that the plots 40 ft. × 5 ft. replicated six times gave the critical difference for significance to be 8·0. Lord [1931] in Ceylon, experimenting with irrigated broadcast rice found that a plot-size of 1/87 acre was the best for field trials under Ceylon conditions. Robertson [1932] found that an error of 2·72 per cent was obtained in a paddy experiment using six repetitions with 1/33 acre per plot. Chien Liang Pan [1935] demonstrated that single-row plots as small as 14·2 feet with 1·5 feet interspace between them were more efficient than increasing length of row in an early variety and the opposite in a late one.

II. Material and methods

From a study of the several uniformity trials conducted in the several countries of the world it is found that the error due to soil heterogeneity may be minimised in the following ways :—

(*a*) by replication (which also secures certain validity),
(*b*) by use and arrangement of blocks (called 'local control' by Fisher) and
(*c*) by using a suitable plot-size and shape.

In the present paper an attempt is made to study the most suitable experimental plot-size at the Rice Research Station, Berhampur (Madras), for experiments on paddy. An area of 0·50 acres was chosen and it was harvested in 512 small plots of 5 ft. × 5 ft. in 1933-34 and also in 1934-35 and the weight of grain from each ultimate unit was recorded.

The yields of the ultimate units were grouped in different ways (1, 2, 3, 4, 6 and 8 units along the columns with 1, 2, 4 and 8 units along the rows or cuts) to give plots of different sizes and shapes. The standard deviations of single plot were calculated for these arrangements and expressed as percentage of the respective means. Assuming a hypothetical number of eight treatments to be compared in the fields, blocks were formed for different plot-sizes. The plots were compounded to form blocks in two different ways and the analysis of variance was used to separate the total 'sum of squares' in each combination and it was seen that a good portion of the variance due to soil heterogeneity has been removed by the 'block' method giving a smaller estimate of error. The standard deviations of the several combinations after elimination of block variance are expressed as percentages of the respective means and these are given in Table I (*a*) and (*b*).

Table I

Standard deviation of a single plot as per cent of mean after elimination of block variance

Columns	Rows						
	1	2	3	4	6	8	16
	(*a*) 1933-34						
1	7·40 7·30	6·03 6·03	5·34 ..	5·24 4·77	5·00 ..	4·15 4·18	3·48 3·53
2	6·06 5·26	5·42 4·60	5·28 ..	4·56 3·80	4·69 ..	3·83 3·26	
4	4·95 4·55	4·61 4·03	4·57 ..	3·91 3·42	4·10 ..	3·98 3·41	
8	4·61 3·79	3·92 3·37	3·88 ..	3·32 2·54	.. ..	.. ..	
16	2·72 ..						

TABLE I—*contd.*

Columns	Rows						
	1	2	3	4	6	8	16
	(*b*) 1934-35						
1	9·48 9·35	8·71 8·25	6·95 ..	6·83 6·52	5·75 ..	5·40 4·59	4·62 4·36
2	8·28 6·59	6·89 5·43	7·00 ..	6·62 4·41	6·45 ..	6·27 4·15	
4	6·87 5·40	6·54 4·39	6·34 ..	6·25 4·21	5·57 ..	5·48 3·72	
8	5·86 4·28	5·31 3·31	4·6 ..	4·87 2·57	.. ..	.. ..	
16	3·91 ..						

Top figures indicate standard deviation when blocks are formed column-wise.

Bottom figures indicate standard deviation when blocks are formed row-wise. From the table it is seen that (*i*) plots having greater length along the columns (along the fertility gradient) have smaller errors than those of the same size running along the rows ; (*ii*) there are only small differences in the coefficient of variation for the same plot-size with different shapes.

It is thus seen that wherever possible the plots should be run along the fertility gradient and the blocks compounded at right angles to these. The shape of the plot is not of great concern and may be chosen as convenient.

With regard to the plot-size a consideration arises, *e.g.*, the availability of the land. Given a certain area, it is the general experience that a large number of small plots always secures a lower error than a smaller number of larger plots up to a certain limit of number and size. " The relative precision of one plot size with variance A to another with variance B, is B/A. But if the size of the first plot is n times the second and if therefore it can permit only $1/n$ times the replications which the latter can admit, the relative precision of the first plot to the second is Bn/A." [Sayer *et al.*, 1936]. Table II (*a*) and (*b*) gives the relative precision of different sizes of plots taking the precision of the smallest plot-size to be 100.

TABLE II

Relative precision of different plot-sizes corrected for replication

	1	2	4	8
		(a) 1933-34		
1	100	73·3	58·6	37·1
2	98·1	62·8	46·1	31·3
4	64·4	41·3	28·3	14·3
8	46·4	23·3	26·4	..
		(b) 1934-35		
1	100	64·3	54·6	51·9
2	100·6	74·2	56·2	27·9
4	74·9	56·7	38·4	20·2
8	64·6	50·0	40·5	..

From the figures in Table II (*a*) and (*b*) it is seen that from the theoretical standpoint, a plot 5 ft. × 5 ft. should be the best for experiments where a small area is available. But obviously the most desirable size of a plot cannot be determined from this quantity of ' efficiency ' alone. This unit of 5 ft. × 5 ft. is so small to experiment with that any small loss of yield in this area by way of uneven ' stand ', a slight attack of pest or disease will magnify the error very much and vitiate the results. It is seen from Table II (*a*) and (*b*) that the efficiency of area 20 ft. × 5 ft. is 74·9 and 64·4 respectively in the two years and this area is pretty big to minimise the influence of accidental and uncontrollable errors and at the same time small enough to secure uniformity of conditions. As such it may be safely recommended for use in experiments with transplanted rice. With six replications and eight treatments the critical difference works out to 5·6 and 6·6 per cent in the two years and this difference is appreciable enough for differentiating between the merits of treatments.

It will be interesting to see how the standard error is affected by including different number of treatments in a block. Using four treatments to a block as a hypothetical case, the standard errors for different sizes for 1934-35 are worked out and are compared with standard errors where eight treatments are included.

TABLE III

Standard errors as percentage of mean with 4 and 8 treatments in a block (1934-35)

Plot-size	8 treatments in a block	4 treatments in a block
ft.		
5 × 5	9·35	9·17
5 × 10	8·25	9·17
5 × 20	6·52	5·42
5 × 40	4·59	5·15
5 × 80	4·36	3·89
10 × 5	6·59	6·36
10 × 10	5·43	5·39
10 × 20	4·41	4·00
10 × 40	4·15	3·40
20 × 5	5·40	5·02
20 × 10	4·39	4·39
20 × 20	4·21	3·37
20 × 40	3·72	2·94
40 × 5	4·28	4·14

It will be seen that though the errors are smaller in the case of four-treatment blocks in many of the sizes these differences are not appreciable.

III. SUMMARY

1. A uniformity trial was conducted at the Rice Research Station, Berhampur, for two years on the same field with 512 plots of 5 ft. × 5 ft. Various combinations were tried from these and the results were worked out by the analysis of variance method taking eight hypothetical treatments to a block.

2. The size of the plot recommended was 20 ft. × 5 ft. where land is limited and a replication of six was found to be adequate for differentiating between merits of treatments.

3. The error was reduced by elongating the plots along the fertility gradient and by compounding the blocks at right angles to it.

2. Correlation of some characters on yield in rice

Yield has been shown by many workers to be complex and as such is found to be not adapted to a simple analysis. No one growth character is seen to be wholly responsible for the eventual yield. In fact, it is a function—it may be a complex type—involving unknown parameters of the metabolic activity of the individual organisms. Plants of the same strain grown under identical conditions do not generally give identical yields. A comparative study of the root-system and the metabolic activities of assimilation and transpiration of the races that compose a certain group of plants brings us into a better understanding of the differences of plant yields ; but the simpler methods of studying the visible yield attributes—such as number of ears per plant, length of ear, density of the ear, height, etc.—also have a certain importance in the study of the effects of some of these characters on yield in rice.

In 1933 in the course of plant-breeding work by the methods of selection, a number of cultures of different habit, growth were isolated in the different varieties. The average yield per plant of all the cultures in each variety together with the mean number of grains per ear were studied and simple correlations between these characters are worked out and given in Table IV.

In 1934, one culture from each of the following varieties of *boroponko, bayyahunda* and *ratnachudi* and the strain GEB 24 were grown under ordinary manured conditions in Berhampur (Ganjam). From about 2,500 plants that were grown, 300 plants were marked at random ; and the number of tiller-bearing earheads per plant was counted in the field just before harvest. (Plants which had more spacing by the death of surrounding plants and the border row plants were omitted). Each plant was harvested (all the earheads only) in paper covers with proper labels. In the laboratory the length of all the ears in each plant was measured and the number of grains in each earhead was counted. As there was not much difference in the period of flowering between the individual plants in each of the cultures, duration was not taken into consideration. Thus measurements of the following characters for each plant were recorded.

1. Yield per plant.
2. Number of tiller-bearing earheads.
3. Average length of earheads.
4. Average number of grains per earhead,

The total coefficients of correlation between any two of the characters measured or the 300 plants in each of the cultures were worked out and are given in Table V.

TABLE IV

Total correlation coefficients

Symbol	Characters correlated	*Boroponko* cultures	*Bayyahunda* cultures	*Ratnachudi* cultures
r_{12}	Yield and number of tillers per plant. .	+0·540±0·027	+0·128±0·038	+0·232±0·038
r_{13}	Yield and average length of earhead .	+0·321±0·033	+0·290±0·037	+0·111±0·039
r_{14}	Yield and average number of grain per ear . . .	+0·184±0·037	+0·160±0·039	+0·100±0·039
r_{23}	Number of tillers and length of ear .	+0·049±0·049	—0·150±0·040	+0·083±0·040
r_{24}	Number of tillers and average number of grains per ear . .	+0·002±0·050	—0·360±0·034	+0·043±0·040
r_{34}	Length of ear and average number of grains per ear .	+0·347±0·034	+0·250±0·038	+0·250±0·038

TABLE V

Total correlation coefficients in several cultures

Symbol	Characters correlated	B P 391	B H D 1608	R C H 1243	G E B 24	Transformed 'z'	r
r_{12}	Yield and number of tillers	+0·808±0·020	+0·833±0·018	+0·875±0·014	+0·791±0·220	+1·187±0·058	+0·83±0·018
r_{13}	Yield and average length of ear	+0·234±0·054	+0·230±0·055	+0·221±0·055	+0·442±0·046	+0·291±0·058	+0·28±0·052
r_{14}	Yield and average number of grains per ear	+0·359±0·050	+0·495±0·044	+0·264±0·054	+0·495±0·044	+0·433±0·058	+0·405±0·048
r_{23}	Number of tillers and length of ear	—0·024±0·058	+0·150±0·056	—0·03±0·058	+0·195± ·056	+0·074±0·058	+0·07±0·057
r_{24}	Number of tillers and average number of grains per ear	+0·069±0·057	+0·127±0·057	—0·079±0·057	+0·175±0·056	+0·073±0·058	+0·06±0·058
r_{34}	Length of ear and number of grains per ear	+0·729±0·027	+0·723±0·027	+0·613±0·036	+0·738±0·026	+0·875±0·058	+0·71±0·028

It is seen from the above table that the correlations show a marked similarity in al the varieties when a particular set of characters is concerned. Thus the yield per plant shows a very high association with number of tillers and a smaller one with length of earhead and the number of grains per ear. The higher association of yield with length of ear and number of grains in GEB 24 is due to the equality of all the earheads in a plant—a characteristic of supreme agronomic value in a variety.

Individual regression equations of yield on the several characters are set out in Table VI.

TABLE VI

Simple regression equations of yield (x_1) on other characters

Strain	Mean number of productive tillers (x_2)	Mean length of earhead (x_3)	Mean number of grain per ear (x_4)
B P 391 .	$x_1=2{\cdot}009x_2+0{\cdot}42$	$x_1=1{\cdot}054x_3-8{\cdot}54$	$x_1=0{\cdot}113x_4+2{\cdot}34$
B H D 1608 .	$x_1=2{\cdot}828x_2-0{\cdot}42$	$x_1=1{\cdot}2x_3-12{\cdot}54$	$x_1=0{\cdot}14x_4+0{\cdot}03$
R C H 1243 .	$x_1=2{\cdot}572x_2+1{\cdot}15$	$x_1=1{\cdot}18x_3-8{\cdot}47$	$x_1=0{\cdot}063x_4+7{\cdot}9$
G E B 24 .	$x_1=1{\cdot}532x_2+1{\cdot}337$	$x_1=1{\cdot}63x_3-22{\cdot}816$	$x_1=0{\cdot}113x_4+2{\cdot}34$

The departure of regression from linearity is tested in the case of regression of yield on number of tillers in *ratnachudi*. The correlation ratio of tillers on yield with twelve arrays was found to be + 0·8793 and the total variance was analysed in Table VII.

TABLE VII

Analysis of variance (yield and tillers in ratnachudi)

Due to	Degrees of freedom	Sum of squares	Mean square
Straight line regression . . .	1	602·7373	602·7373
Deviation from regression . . .	10	59·1394	5·9139
Residual	288	7178·83	24·9
Total .	299	7840·71	

It is thus seen that the deviation from regression is not significant by '*z*'-test and that the regression of yield on number of tillers follows the linear law. The partial correlation coefficients were calculated and are set out in Table VIII.

TABLE VIII

Partial correlations

Symbol	B P 391	B H D 1608	R C H 1243	G E B 24
$r_{12\cdot34}$.	+0·879±0·013	+0·935±0·008	+0·969±0·004	+0·820±0·019
$r_{13\cdot24}$.	+0·130±0·057	+0·538±0·041	+0·182±0·056	+0·088±0·058
$r_{14\cdot23}$.	+0·768±0·024	+0·728±0·027	+0·574±0·039	+0·457±0·046
$r_{23\cdot14}$.	+0·351±0·051	—0·608±0·037	—0·157±0·057	+0·237±0·055
$r_{24\cdot13}$.	+0·360±0·051	—0·770±0·024	—0·546±0·042	—0·317±0·052
$r_{34\cdot12}$.	+0·830±0·018	+0·591±0·038	+0·532±0·042	+0·614±0·037

It is interesting to compare the total correlation coefficients and the corresponding partial correlations. The correlations between yield and number of tillers were improved in all the four varieties by the elimination of the other two characters. The relation between yield and length of ear becomes negligible when the influence of the other two characters is eliminated as the number of tillers itself explains a good portion of the variation in yield. Number of grains per ear, however, shows a higher association with yield in the partial correlations. This is a useful property as it shows that the yield increases with density of the earhead, when length of ear and number of tillers remain constant. The partial correlations between number of grains and tillers and the partial correlations between tillers and length of head are negative in three cases. It would therefore seem that too much of increased tiller production is detrimental to earhead length and number of grain per ear and it is therefore not possible to increase the tillering indefinitely without affecting the yield.

Multiple regression equations which will predict the change of the dependent variable by changes on the independent variable have been worked out and are given below.

Multiple regression equations

B P 391 : $x_1 = 1{\cdot}97\,x_2 + 0{\cdot}26\,x_3 + 0{\cdot}081\,x_4 - 14{\cdot}88$
B H D 1608 : $x_1 = 2{\cdot}72\,x_2 + 0{\cdot}46\,x_3 + 0{\cdot}111\,x_4 - 24{\cdot}37$
R C H 1243 : $x_1 = 2{\cdot}65\,x_2 + 0{\cdot}36\,x_3 + 0{\cdot}071\,x_4 - 9{\cdot}11$
G E B 24 : $x_1 = 1{\cdot}63\,x_2 + 0{\cdot}241\,x_3 + 0{\cdot}113\,x_4 - 14{\cdot}88$

The equations are very emphatic in showing the predominant influence of tillering on plant yield. An increase of one tiller per plant increases the yield per plant by $1\frac{1}{2}$ to 3 grams. The influence of increase of length of earhead on yield is less compared with the influence of the number of grains per ear. These facts are very useful in agronomic practice and serve as useful guide in selection work. Tillering is an elastic character and can be influenced by judicial manuring and cultural practices.

The calculated yields from the above regression equations gave a good fit with the observed, the very high and low values of yield predicted from the equations being very near the observed yields.

The multiple correlation coefficient, which measures the combined importance of the several independent factors as a means of explaining the differences in the dependent factor—here the yield—is calculated and given in Table IX.

TABLE IX

Multiple correlation coefficients

Nature of correlation	B P 391	B H D 1608	R C H 1243	G E B 24
$R_{1\cdot34}$ . . .	0·3610	0·5259	0·2757	0·5259
$R_{1\cdot23}$ . . .	0·7700	0·8400	0·8930	0·8560
$R_{1\cdot24}$ . . .	0·8408	0·9198	0·9069	0·8780
$R_{1\cdot234}$ . . .	0·8520	0·9284	0·9295	0·8786

$R_{1\cdot234}$ has the highest value but its differences from $R_{1\cdot23}$ and $R_{1\cdot24}$ are small. That is a good deal of variation in yield is explained primarily by the inclusion of tillers and secondarily by the length of ear and number of grain. $R_{1\cdot34}$ is only 0·361 showing that only a little variation in yield could be explained by the combined action of length of ear and number of grains.

Correlations have also been worked out in *ratnachudi*, *bayyahunda* and GEB 24 under unmanured conditons. The order of correlations observed in the manured series was maintained, *i.e.* the correlation between yield and tillers was the highest followed by that between yield and number of grain and yield and length of ear ; but the length of ear and number of grains per ear had a greater influence on yield under no-manured conditions.

It is obvious that yield of rice per acre, in other words per unit area, is dependent broadly upon the product of the two main components, *e.g.*, number of tillers per unit area and the average yield per ear. The trend of the correlations has shown that the plant yield is highly associated with number of tillers per plant, or in other words the yield is greatly dependent upon the number of ears per unit area. Thus it must be the aim of every agriculturist to augment the number of tillers per unit area by means of optimum spacing, number of plants per hole, vigorous seedlings, by better cultural practices and judicial manuring. Of secondary importance in determining the yield is the number of grains per ear and then length of ear, *i.e.*, the density of the earhead plays an important part in selection work. It is necessary to see that the variation between earheads of the same plants is not much ; ‘ chaffiness ’ or ‘ sponginess ’ lessens the value of a strain.

I. Review of previous work

Workers on rice in different parts of the country have found different degrees of correlation between yield and some characters. All of them have obtained a fairly high amount of association between yield and number of ears per plant. Bhide and Bhalerao [1927] found a coefficient of $+ 0\cdot71$ to $+ 0\cdot80$ between yield per plant and tillering; tillering and length of main panicle has a correlation of $+ 0\cdot11$ to $+ 0\cdot22$; they found a negative correlation between tillers and weight of panicle. Copeland [1924] summarises the work of correlations in rice in his work on "Rice". Recently Mitra [1932] working upon rices of Assam worked out some correlations between yield and other characters.

II. Summary

1. Total, partial correlations and multiple regression equations between yield and three characters (*viz.*, number of tillers per plant, mean length of ear and number of grains per ear) were calculated in three important paddy varieties of the Ganjam and GEB 24.

2. Number of tillers bore the highest correlation with yield both total and partial, followed by number of grain per ear and length of ear.

3. Under no-manure conditions the number of grain and length of ear exert a little higher influence than under manured conditions.

III. Acknowledgments

The work was done with the help of the research grant made by the Imperial Council of Agricultural Research. It is a great pleasure to acknowledge the help received during the computation of results under the guidance of Prof. P. C. Mahalanobis of the Statistical Laboratory, Calcutta. My special thanks are due to Rao Bahadur M. Vaidyanathan, M.A., Statistician to the Imperial Council of Agricultural Research, who went through the manuscript and offered valuable criticism and also to Mr. K. Ramiah, M.Sc., Dip. Agri. (Cantab.), L. Ag., Paddy Specialist, Coimbatore, and to Mr. S. Jobitha Raj, B.A., Superintendent, Rice Research Station, Berhampur, who suggested and guided the work during the different stages.

REFERENCES

Bhide, R. K. and Bhalerao, S. G. (1927). *Mem. Dept. Agric. Ind.* (*Bot. Ser.*) **14**, No. 7.

Chien, Liang Pan (1935). *J. A. Soc. Agric.* **27**, 279-285.

Copeland, E. B. (1924). 'Rice', McMillan & Co., London.

Lord, L. (1931). *J. Agric. Sci.* **21**, 178-186.

Mad. Agric. Stn. Reports. 1926-27.

Mitra, S. K. (1932). *Ind. J. Agric. Sci.* **2**, 571-606.

Parnell, F. R. (1919). *Agric. J. Ind.* **14**, 747-756.

Ramiah, K. (1931-32). *Mad. Agric. Stn. Reports, 1931-32*, p. 377-378.

Robertson, H. F. (1932). *Ind. J. Agri. Sci.* **2**, 53-58.

Sayer, W.; Vaidyanathan, M. and Subramonia Iyer, S. (1936). *Ind. J. Agric. Sci.* **6**, 684-715.

AGRICULTURAL METEOROLOGY

THE PREDICTION OF THE MINIMUM TEMPERATURE ON CLEAR DAYS AT SELECTED STATIONS IN INDIA*

BY

M. NARASIMHAN, M.A.

Research Student, Agricultural Meteorology Section

AND

L. A. RAMDAS, M.A., PH.D.

Agricultural Meteorologist, Meteorological Office, Poona

(Received for publication on 14th May 1937)

(With seven text-figures)

INTRODUCTION

It is well known that temperature is an important factor in the control of the growth and yield of crops and that extremes of heat and cold are injurious to plants. In a recent paper Ramdas [1935] has discussed the problems of frost-hazard in India and there reminded us that the frequency of abnormally low temperatures associated with frost is negligible in South India (south of latitude 18°N.) but increases with latitude as one enters Central and North India, the maximum frequency being over the Punjab and Kashmir area. In the present paper an attempt is made to investigate the possibility of predicting, by statistical methods, the minimum temperature attained by the air at four feet above ground during clear weather from a knowledge of the maximum temperature and vapour pressure recorded on the previous afternoon.

In Section I a brief account of the radiation processes responsible for nocturnal cooling is given. In the next section the results of the statistical analysis of data of nineteen representative stations in India are discussed. These results suggest that the minimum temperature can be expressed as a function of the maximum temperature and the pressure of water vapour of the previous afternoon. The forecasting formulæ obtained for Calcutta and Lahore are found, on a detailed examination, to satisfy the 'performance test' [Normand, 1932] very satisfactorily.

I. NOCTURNAL RADIATION AND COOLING OF THE AIR LAYERS NEAR THE GROUND

The processes involved in the cooling of the earth's surface and of the air layers near the ground have been discussed in a series of papers based on experimental work carried out at Poona during the past few years [Ramdas and Atmanathan, 1932; Ramanathan and Ramdas, 1935; Raman, 1935; 1936; Ramdas, Sreenivasayya and Raman, 1937]. The earth's surface emits heat radiation practically like a black body at the surface temperature. At sunset insolation is withdrawn and the surface of the ground and the air layers near it begin to cool rapidly as a result of loss of heat by radiation. It must be remembered that the atmosphere always contains sufficient water vapour to absorb and return some of this radiation to the surface of the ground. The heat radiation S coming from the water vapour in the atmosphere can be expressed as:

---

*This investigation was made in the Agricultural Meteorology Section (Meteorological Office, Poona) financed by the Imperial Council of Agricultural Research.

$$\frac{S}{\sigma T^4} = A - B \times 10^{-\gamma e} \quad \ldots\ldots\ldots\ldots \quad (1)$$

where T is the temperature in degrees absolute and e the aqueous vapour pressure (in mm. of mercury) of the air at the place of observation, σT^4 is the black body radiation at temperature T and A, B and γ are constants. From actual measurements of S carried out at Poona and elsewhere it has been shown that A, B and γ have the mean values of 0·77, 0·28 and 0·074 respectively. S and σT^4 are expressed in gramme-calories per square centimetre per minute.

$\frac{S}{\sigma T^4}$ gives the ratio of the heat radiation actually received per minute by unit area of a horizontal surface from the whole of the sky on a clear night to the heat radiation which would have been received per minute from the sky if it were a perfect black body at $T°A$. Equation (1) gives the above ratio as a function of the vapour pressure (e) at the surface. This really amounts to expressing $\frac{S}{\sigma T^4}$ as a function of the total precipitable water vapour (w) contained in a vertical cylinder one sq. cm. in cross-section and extending upwards to the limit of the earth's atmosphere, for, according to Hann, w may be put equal to $0·21\,e$ approximately. For a fuller discussion of the subject a reference is invited to the paper by Ramanathan and Ramdas [1935].

For a given temperature T the value of S, which is one of the factors setting a limit to the rate of nocturnal cooling, increases as e increases. The curve in Fig. 1 (*a*) shows how $\frac{S}{\sigma T^4}$ varies with e. When e approaches zero $\frac{S}{\sigma T^4}$ tends to attain a minimum value of 0·49 whereas when e becomes large $\frac{S}{\sigma T^4}$ approaches 0·77. Other conditions remaining the same we should therefore expect nocturnal cooling to be maximum on days when the atmosphere is dry and minimum when the atmosphere contains a large amount of precipitable water vapour.

The relation between the diurnal range of air temperature at four ft. during clear and calm weather and the pressure of water vapour at the same level, when the maximum temperature is of the order of 30·5°C. (87°F.), has been worked out from the frequencies of different magnitudes of the diurnal range of temperature associated with different values of the vapour pressure. In order to get a sufficient range of vapour pressure the data of seventeen typical stations in India were pooled together. The mean values of the diurnal range of temperature $\Delta\ T$ associated with different values of the vapour pressure are plotted in Fig. 1 (*b*). The points fall more or less on a smooth curve. From this curve one sees that the maximum value of $\Delta\ T$ is of the order of 25°C. when *e*, the vapour pressure, approaches zero whereas the value of $\Delta\ T$ is only 6°C. when *e* is of the order of 25 mm.

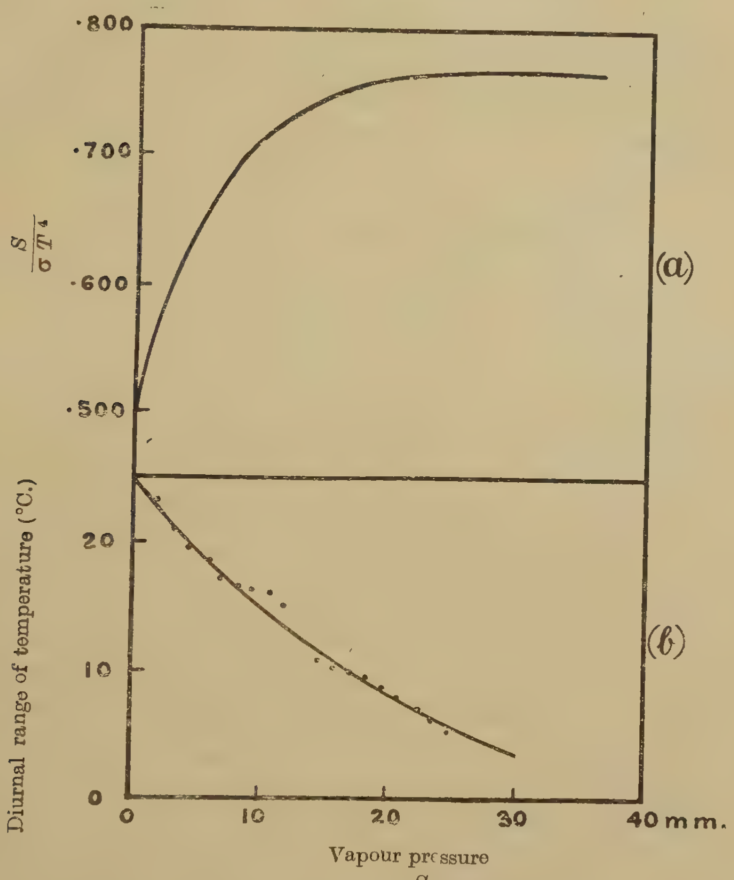


FIG. 1. (*a*) Showing the variation of $\frac{S}{\sigma T^4}$ with vapour pressure; (b) Mean values of the diurnal range of temperature in relation to vapour pressure.

It may be mentioned that $\Delta\ T$, besides being a function of the intensity of cooling which tends to stratify the air layers near the ground, is also influenced by the velocity of air movements and consequent turbulence and mixing of the air layers. Considering that data of a number of stations with varying conditions of exposure have been considered, it is indeed remarkable that Fig. 1 (*b*) brings out so clearly the importance of radiation as influenced by the water content of the atmosphere. The initial constant temperature of 30·5°C. at the maximum temperature epoch was used in arriving at Fig. 1 (*b*) because it was for this temperature that the fullest information of ΔT was available for a wide range of e, *viz.* 0 to 25 mm.

The influence of turbulence and radiation in controlling the stability and cooling of the air layers near the ground after sunset is being investigated at the Central Agricultural Meteorological Observatory at Poona by taking detailed psychrometric and wind observations at short intervals of time and height above ground during one hour preceding and two hours succeeding sunset during the winter season. These data will be discussed in a forthcoming paper.

II. Statistical analysis of data

Correlation of maximum temperature and vapour pressure in the afternoon with the minimum temperature of the succeeding morning

There is a considerable amount of literature on the subject of predicting the minimum temperature from a knowledge of temperature and humidity of the previous evening.* It is usual in such investigations to use the relative humidity (*H* per cent), the wet-bulb temperature or the dew point in conjunction with the evening dry-bulb temperature.

Recent investigations on radiation indicate, however, that so far as the humidity factor is concerned the vapour pressure is the most relevant element to use, because it indicates directly the water-vapour content of the air layer near the ground which can be expressed as a function of the total water content of the atmosphere (as indicated earlier). In the present paper the vapour pressure and the maximum temperature recorded in the afternoon and the minimum temperature of the next morning are the factors whose inter-relations are investigated.

Data

From an examination of the normal climatological records of the selected stations (Fig. 2) it is easy to pick out comparatively clear months. The months selected are indicated in Table I below :—

---

*For previous literature on this subject please see p. 132 of " Physical and Dynamica Meteorology " by D. Brunt [1934], and a paper on the " Correlation between Frost and preceding Meteorological Conditions " by Barkat Ali and Naqvi, *Ind. J. Agric. Sci.* 1. Part 6, Dec. 1931.

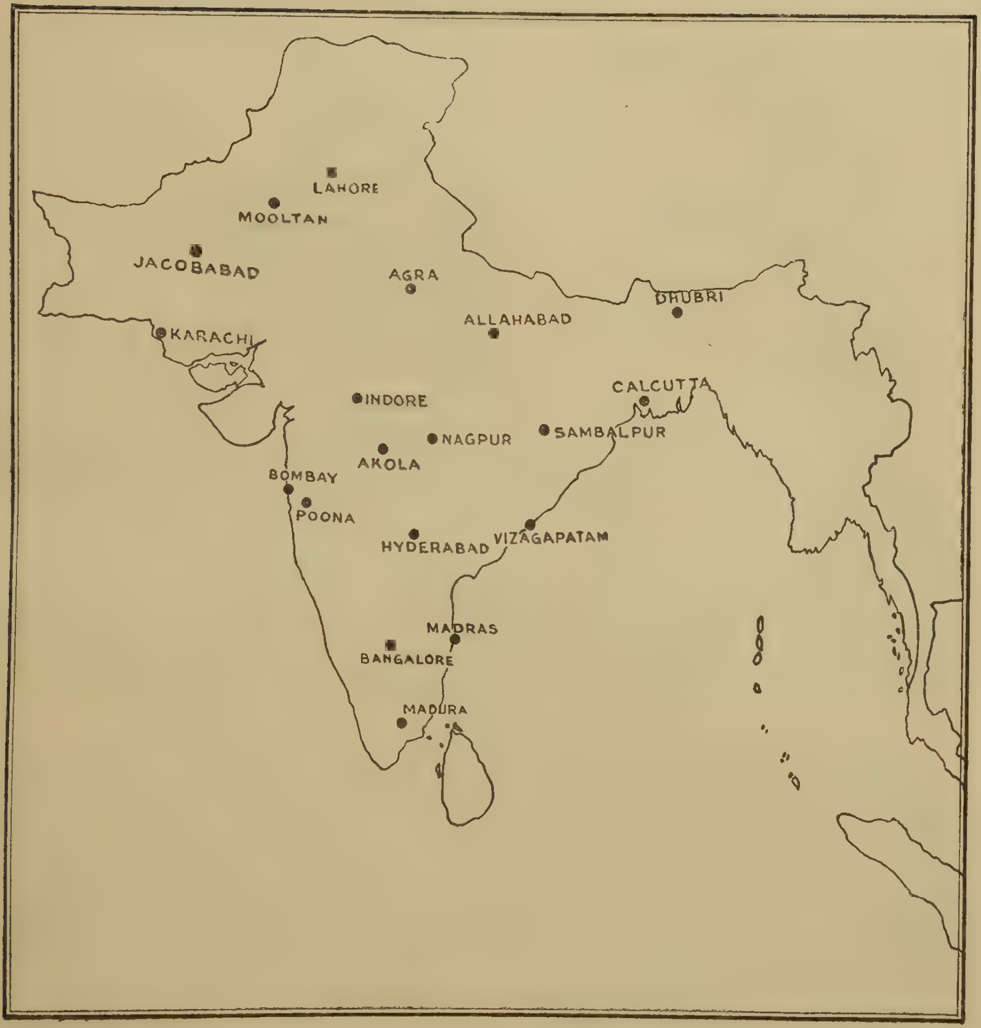

LAHORE
MOOLTAN
JACOBABAD
AGRA
ALLAHABAD
DHUBRI
KARACHI
INDORE
CALCUTTA
NAGPUR
SAMBALPUR
AKOLA
BOMBAY
POONA
HYDERABAD
VIZAGAPATAM
MADRAS
BANGALORE
MADURA

FIG. 2. Map of India showing selected stations.

TABLE I

Name of station	Months
1. Calcutta	November, December, January (1931-33).
2. Sambalpur	November, December (1933-35).
3. Dhubri	November, December (1933-35).
4. Allahabad	November, December (1934-35).
5. Agra	January, February (1930-32).
6. Karachi	October, November (1933-35).

TABLE I—*contd.*

Name of station	Months
7. Indore	January (1930-35).
8. Nagpur	January (1930-35).
9. Akola	January (1930-35).
10. Bombay	January (1930-35).
11. Poona	January, February (1933-35).
12. Hyderabad (Deccan)	February, March (1933-35).
13. Bangalore	February, March (1933-35).
14. Madura	February, March (1933-35).
15. Madras	February, March (1933-35).
16. Vizagapatam	February, March (1933-35).
17. Jacobabad	May, June (1933-34).
18. Multan	September, October (1933-34).
19. Lahore	January (1927-32).

The daily records of the individual stations for the months noted against them were examined and the records of the afternoon maximum temperature and vapour pressure* (recorded at 17 hours) preceding clear nights (as judged from total absence of clouds at 17 † hours) as well as the minimum temperature recorded on the succeeding morning were tabulated for examination. The data of 100 clear days during the clear months of a few years were thus selected. In the case of Hyderabad (Deccan) and Madura, 17 hours observations were not available and so the morning 8 hours observations of vapour pressure and cloudiness were considered.

Dot diagrams

Before proceeding to calculate the correlation coefficients between the elements concerned, dot diagrams of (*a*) the vapour pressure at 17 hours against the next morning minimum temperature and (*b*) the maximum temperature of the afternoon against the next morning minimum temperature respectively were prepared for each station. These diagrams indicated relationship between these elements in the case of most of the stations. As examples, the diagrams in the case of Akola Figs. 3 (*a*) and (*b*), Bombay (Colaba) Figs. 4 (*a*) and (*b*), Madura Figs. 5 (*a*) and (*b*) and Calcutta Figs. 6 (*a*) and (*b*) are reproduced. It is sufficient to bear in mind that the closer the dots lie together along a curve the closer will be the relationship between the elements.

---

*Maximum temperature is attained usually some time between 14 and 16 hours I. S. T. The diurnal variation of vapour pressure on clear days is usually small and the 17 hours observation of this element would be more or less representative of conditions during the afternoon and night.

†There may be occasions during the clear season when the skies are clear at 8 hours and 17 hours, but become clouded temporarily during the night. On such nights the minimum temperature will be higher than the value predicted from conditions during the preceding afternoon.

Similarly, the actual minimum temperature will deviate from the predicted value, if there is any temporary disturbance during the night resulting in the replacement of one air mass by another with higher or lower temperature and water content.

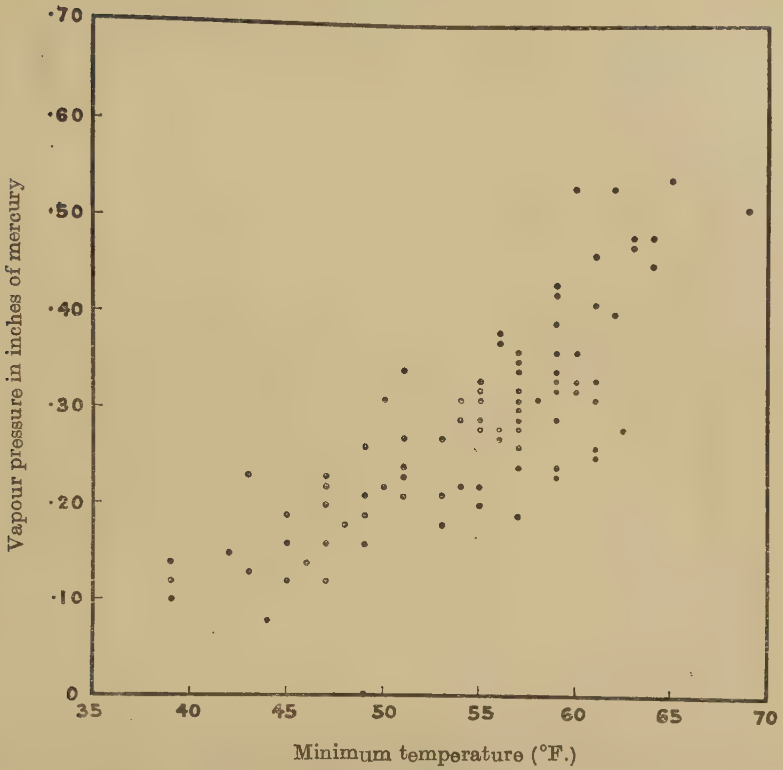


FIG. 3 (*a*). Vapour pressure at 17 hrs. against next morning minimum temperature at Akola.

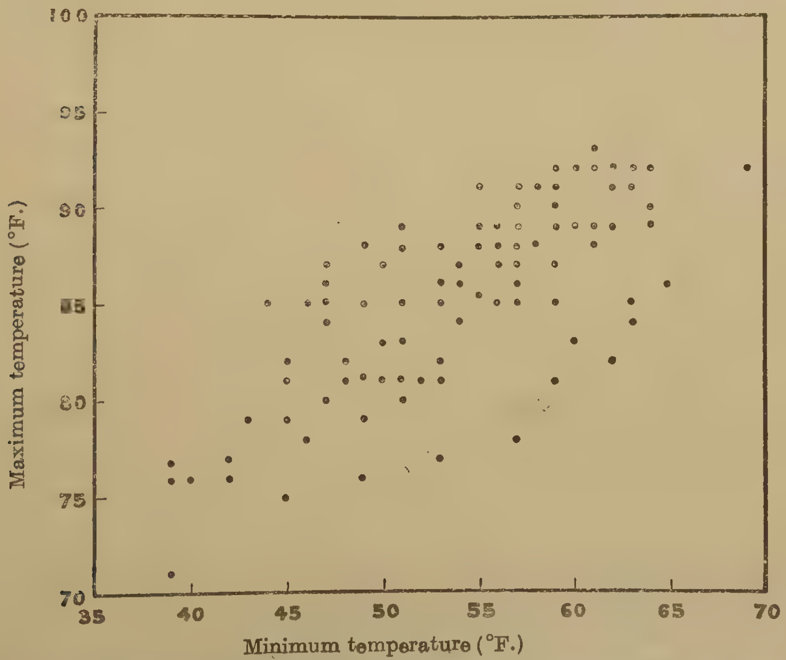


FIG. 3 (*b*). Maximum temperature against next morning minimum temperature at Akola.

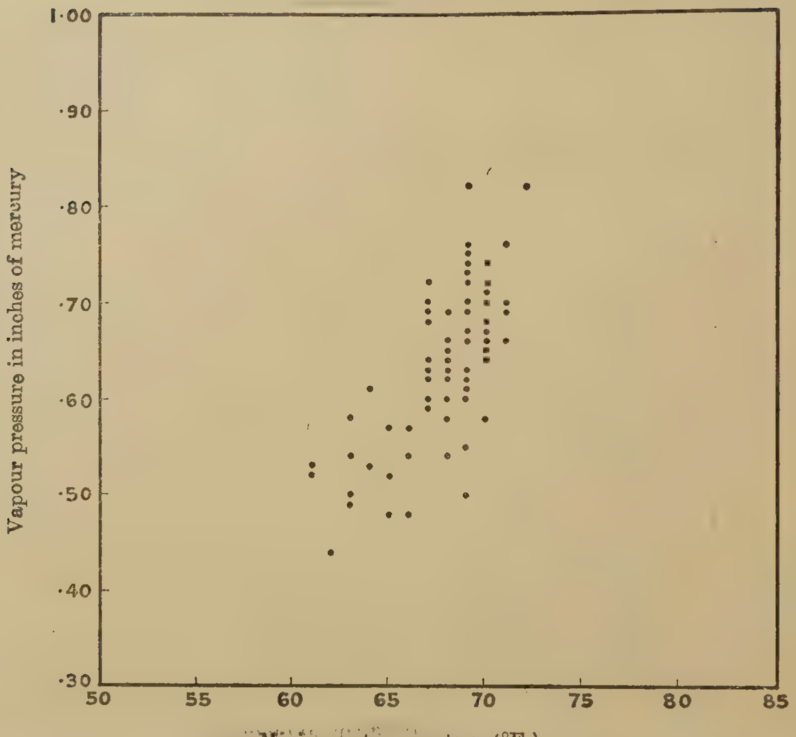


FIG. 4 (*a*). Vapour pressure at 17 hrs. against next morning minimum temperature at Colaba (Bombay).

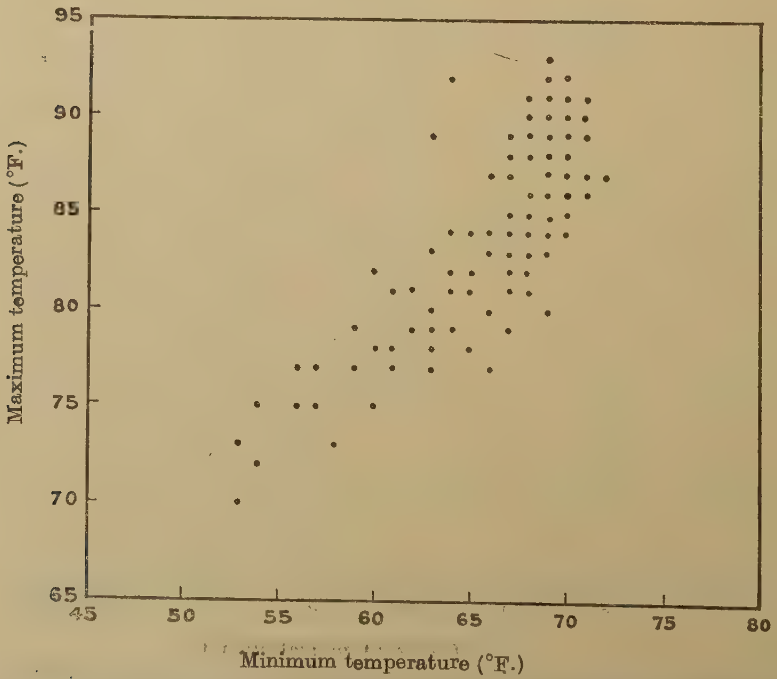


FIG. 4 (*b*). Maximum temperature against next morning minimum temperature at Colaba (Bombay).

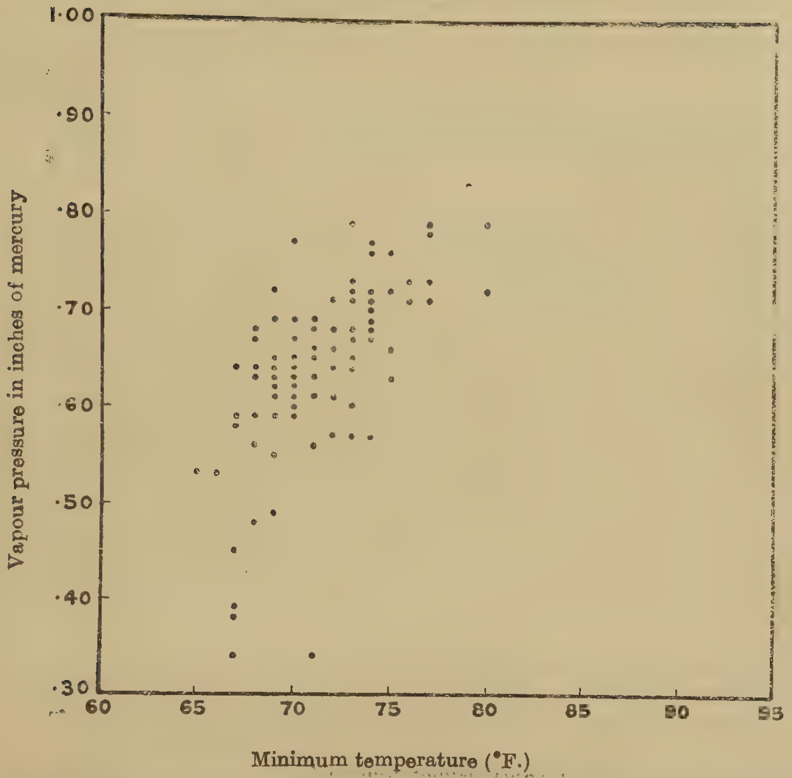


FIG. 5 (*a*). Vapour pressure at 17 hrs. against next morning minimum temperature at Madura.

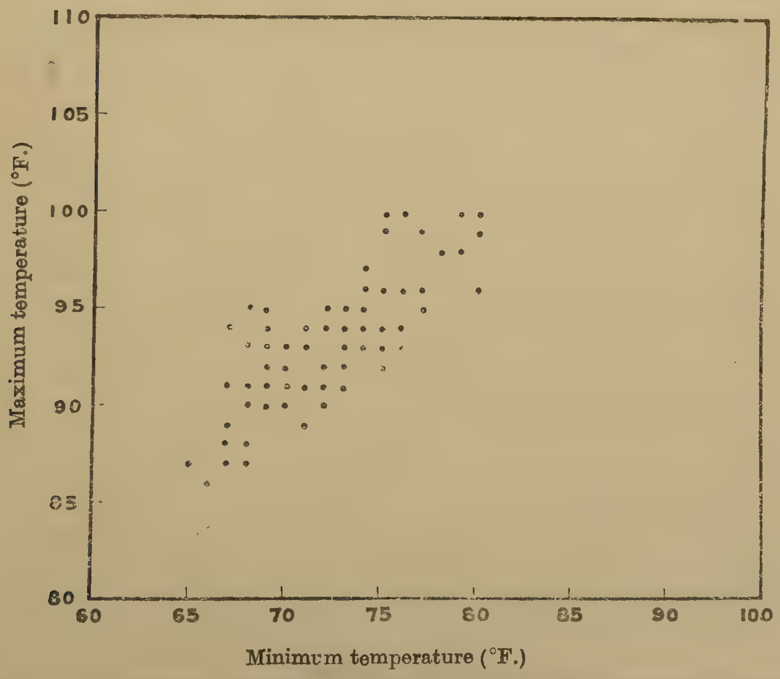


FIG. 5 (*b*). Maximum temperature against next morning minimum temperature at Madura.

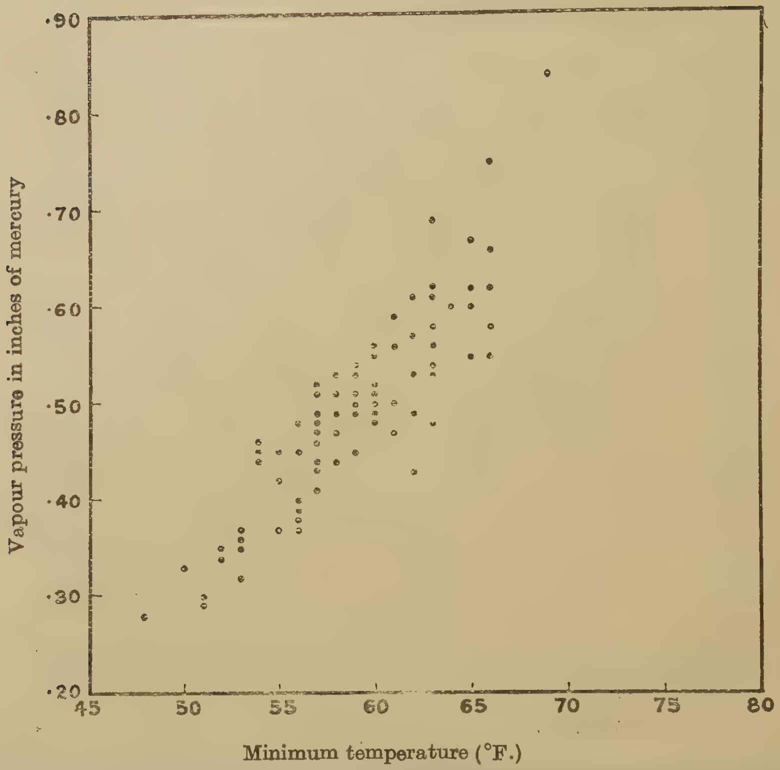


FIG. 6 (*a*). Vapour pressure at 17 hrs. against next morning minimum temperature at Calcutta.

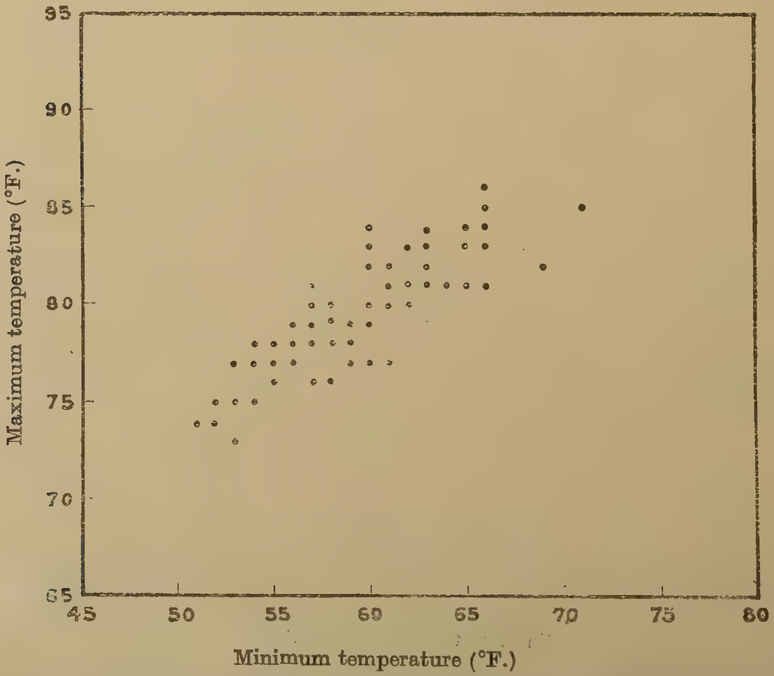


FIG. 6 (*b*). Maximum temperature against next morning minimum temperature at Calcutta.

The mean values of the maximum temperature, minimum temperature and vapour pressure, together with their standard deviations (S. D.) are given in Table II.

TABLE II

Name of station	Maximum temperature (°F.)		Vapour pressure in inches of mercury		Minimum temperature (°F.)	
	Mean	S. D.	Mean	S. D.	Mean	S. D.
Calcutta	79·5	2·9	0·49	0·10	58·9	4·2
Sambalpur	84·0	3·3	0·43	0·07	54·3	4·4
Dhubri	75·7	3·7	0·53	0·08	59·1	4·7
Allahabad	81·0	5·3	0·37	0·09	52·7	5·2
Agra	75·9	5·2	0·26	0·12	45·1	5·1
Karachi	86·3	3·1	0·72	0·16	70·1	3·6
Indore	81·2	4·7	0·20	0·06	49·3	5·4
Nagpur	84·3	4·3	0·29	0·08	53·9	4·8
Akola	86·5	4·4	0·28	0·14	54·7	6·2
Bombay	86·3	3·8	0·65	0·08	68·1	2·2
Poona	89·3	3·9	0·23	0·08	51·4	5·2
Hyderabad (Deccan) . .	92·2	3·7	0·46	0·11	64·7	4·3
Bangalore	89·3	3·4	0·28	0·07	62·1	4·1
Madura	92·3	3·1	0·64	0·09	71·3	3·3
Madras	86·7	2·3	0·73	0·09	69·1	3·2
Vizagapatam . . .	83·8	2·6	0·77	0·10	70·2	3·7
Jacobabad	109·1	5·7	0·55	0·15	82·9	4·6
Multan	98·8	4·7	0·71	0·23	70·7	7·8
Lahore	68·4	4·7	0·29	0·06	40·5	4·4

The values of the correlation coefficients between (*a*) maximum and minimum temperatures, r_{XN}, (*b*) minimum temperature and vapour pressure, r_{NV} and

(c) maximum temperature and vapour pressure, r_{XV} are given for the different stations in Table III.

TABLE III

Station	Correlation coefficients		
	r_{XN}	r_{NV}	r_{XV}
Calcutta	0·85	0·89	0·75
Sambalpur	0·55	0·71	0·44
Dhubri	0·85	0·74	0·56
Allahabad	0·69	0·72	0·50
Agra	0·68	0·51	0·42
Karachi	0·43	0·63	0·17
Indore	0·81	0·61	0·42
Nagpur	0·66	0·73	0·35
Akola	0·67	0·60	0·24
Bombay	0·74	0·64	0·63
Poona	0·68	0·60	0·10
Hyderabad (Deccan)	0·65	0·42	0·08
Bangalore	0·76	0·35	0·07
Madura	0·65	0·63	0·44
Madras	0·55	0·81	0·41
Vizagapatam	0·47	0·77	0·66
Jacobabad	0·55	0·57	0·23
Multan	0·68	0·71	0·45
Lahore	0·52	0·60	0·23

Generally speaking the values of r_{XN} and r_{NV} are fairly high. It may be noted that $r = 0·236$ and $r = 0·198$ correspond to the one per cent and five per cent levels of significance according to Fisher's table. According to Walker's test as extended by Savur and Gopal Rao [1932], the 'five per cent chance' highest value of r in a series of nineteen values due to accident is 0·305. All the values of r_{XN} and r_{NV} are significant even according to this stringent test. The values of r_{XV} in the case of Karachi, Akola, Poona, Hyderabad, Bangalore, Jacobabad and Lahore are insignificant and most of the remaining values are smaller than the corresponding values of r_{XN} and r_{NV}.

Regression equations, of the form $N = b_x X + b_v V +$ constant, were computed for the nineteen stations, expressing the minimum temperature N in terms of the maximum temperature X and vapour pressure V of the previous evening (b_x and b_v being constants). Multiple correlation coefficients were worked out in every case and they were all found to be above 0·7. To be significant, the multiple correlation coefficient should be greater than 0·310 for one per cent level of significance according to Fisher's table. The values of t of the regression coefficients were calculated for all the stations and are given along with the regression equations and multiple correlation coefficients in Table IV. The value of t for the five per cent level of significance is 2. It is interesting to note that all the formulæ in Table IV satisfy these tests of significance.

TABLE IV

Station	Regression equation, $N = b_x X + b_v V +$ constant	Multiple correlation, R	Value of t	
			b_x	b_v
Calcutta	$-2\cdot11 + 0\cdot6123\,X + 25\cdot15\,V$	0·93	7·5	10·5
Sambalpur	$4\cdot52 + 0\cdot4058\,X + 36\cdot51\,V$	0·75	4·1	7·7
Dhubri	$-15\cdot37 + 0\cdot8245\,X + 22\cdot75\,V$	0·91	12·5	7·3
Allahabad	$6\cdot60 + 0\cdot4355\,X + 29\cdot25\,V$	0·81	6·5	7·2
Agra	$0\cdot54 + 0\cdot5467\,X + 11\cdot78\,V$	0·72	7·3	3·5
Karachi	$26\cdot48 + 0\cdot3956\,X + 13\cdot17\,V$	0·72	4·7	8·1
Indore	$-19\cdot76 + 0\cdot7754\,X + 30\cdot50\,V$	0·86	11·8	5·8
Nagpur	$0\cdot79 + 0\cdot5066\,X + 35\cdot89\,V$	0·85	8·0	10·0
Akola	$-18\cdot72 + 0\cdot7797\,X + 21\cdot36\,V$	0·81	9·0	7·6
Bombay	$34\cdot71 + 0\cdot3257\,X + 8\cdot12\,V$	0·78	6·8	3·6
Poona	$-33\cdot44 + 0\cdot8607\,X + 34\cdot71\,V$	0·87	12·6	10·7
Hyderabad (Deccan)	$-8\cdot44 + 0\cdot7249\,X + 13\cdot70\,V$	0·75	9·3	5·4
Bangalore	$-29\cdot91 + 0\cdot9568\,X + 23\cdot46\,V$	0·86	15·1	7·7
Madura	$15\cdot95 + 0\cdot4945\,X + 15\cdot16\,V$	0·76	6·3	5·7
Madras	$20\cdot15 + 0\cdot3547\,X + 24\cdot93\,V$	0·85	4·3	11·8
Vizagapatam	$40\cdot64 - 0\cdot0875\,X + 28\cdot88\,V$	0·77	0·7	9·4
Jacobabad	$36\cdot32 + 0\cdot3450\,X + 16\cdot22\,V$	0·73	6·0	7·1
Multan	$-16\cdot04 + 0\cdot7510\,X + 17\cdot74\,V$	0·82	7·0	7·9
Lahore	$4\cdot96 + 0\cdot3692\,X + 35\cdot48\,V$	0·72	5·7	7·3

Using the regression equation, the values of the minimum temperatures were calculated in the case of Calcutta. The calculated values agree closely with the actuals and are given in Table V. The calculated and the actual minimum temperatures are also shown in Fig. 7. The equations for other stations may be used in a similar way for obtaining calculated values of the minimum temperature.

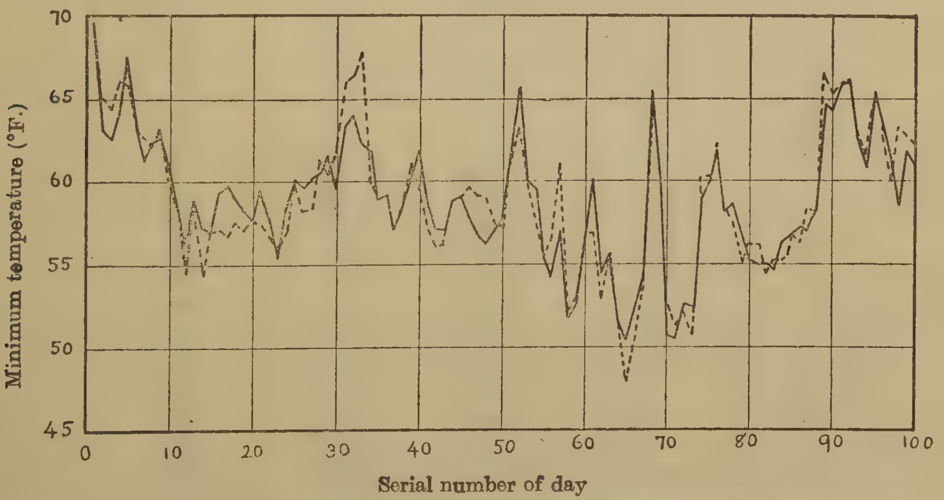


FIG. 7. Actual (------) and calculated (——) values of the minimum temperature at Calcutta on 100 clear days (1931-33)

Table V

Calcutta

	Maximum temperature (°F.)	Vapour pressure (in inches)	Actual minimum temperature (°F.)	Calculated minimum temperature (°F.)	'Actual' *minus* 'calculated' minimum temperature (°F.)
1	82	0·845	69	69	0
2	84	0·548	65	63	2
3	81	0·602	64	63	1
4	81	0·657	66	64	2
5	83	0·754	66	67	—1
6	82	0·620	63	64	—1
7	81	0·560	63	61	2
8	80	0·608	62	62	0
9	81	0·608	63	63	0
10	80	0·551	60	61	—1
11	79	0·495	58	59	—1
12	77	0·455	54	56	—2
13	79	0·489	58	59	—1
14	78	0·463	54	57	—3
15	79	0·430	57	57	0
16	81	0·477	57	59	—2
17	81	0·479	57	60	—3
18	80	0·476	57	59	—2
19	80	0·463	57	58	—1
20	79	0·437	58	57	1
21	79	0·516	57	59	—2
22	80	0·432	57	58	—1
23	79	0·374	56	55	1
24	79	0·470	57	58	—1
25	82	0·479	60	60	0
26	80	0·510	58	60	—2
27	80	0·526	58	60	—2
28	82	0.501	61	61	0
29	84	0·482	60	61	—1
30	83	0·427	62	59	3
31	84	0·552	66	63	3
32	84	0·582	66	64	2
33	83	0·540	63	62	1
34	83	0·521	60	62	—2
35	79	0·502	59	59	0
36	79	0·508	59	59	0
37	79	0·436	57	57	0
38	78	0·494	59	58	1
39	79	0·563	61	61	0
40	82	0·551	60	62	—2
41	79	0·495	57	59	—2
42	78	0·449	56	57	—1
43	77	0·481	56	57	—1

TABLE V—*contd.*

	Maximum temperature (°F.)	Vapour pressure (in inches)	Actual minimum temperature (°F.)	Calculated minimum temperature (°F.)	'Actual' *minus* 'calculated' minimum temperature (°F.)
44	78	0·535	59	59	0
45	78	0·541	59	59	0
46	77	0·508	60	58	2
47	77	0·455	59	57	2
48	77	0·449	59	56	3
49	78	0·475	58	57	1
50	78	0·475	57	58	—1
51	81	0·586	61	62	—1
52	83	0·688	63	66	—3
53	79	0·563	60	60	0
54	78	0·551	58	60	—2
55	77	0·375	55	55	0
56	76	0·381	57	54	3
57	77	0·469	61	57	4
58	74	0·345	52	52	0
59	75	0·358	53	52	1
60	79	0·411	57	56	1
61	81	0·510	57	60	—3
62	78	0·350	53	54	—1
63	79	0·399	56	55	1
64	74	0·300	51	52	—1
65	74	0·284	48	50	—2
66	75	0·333	50	52	—2
67	77	0·374	53	54	—1
68	83	0·669	65	65	0
69	82	0·498	60	60	0
70	73	0·322	53	51	2
71	74	0·297	51	50	1
72	75	0·350	52	53	—1
73	77	0·302	51	52	—1
74	79	0·495	60	59	1
75	79	0·557	60	60	0
76	81	0·567	62	62	0
77	79	0·469	59	58	1
78	79	0·495	57	59	—2
79	78	0·449	55	57	—2
80	77	0·393	56	55	1
81	77	0·399	56	55	1
82	75	0·436	54	55	—1
83	76	0·418	55	55	0
84	77	0·449	55	56	—1
85	78	0·443	57	57	0
86	77	0·481	56	57	—1
87	76	0·488	58	57	1

TABLE V—*concld.*

	Maximum temperature (°F.)	Vapour pressure (in inches)	Actual minimum temperature (°F.)	Calculated minimum temperature (°F.)	'Actual' *minus* 'calculated' minimum temperature (°F.)
88	78	0·495	58	58	0
89	84	0·619	66	65	1
90	84	0·601	65	64	1
91	86	0·619	66	66	0
92	85	0·625	66	66	0
93	84	0·535	63	63	0
94	83	0·492	62	61	1
95	84	0·625	66	65	0
96	83	0·577	63	63	0
97	82	0·548	60	62	—2
98	79	0·483	63	59	4
99	82	0·560	63	62	1
100	81	0·532	62	61	1

Performance test

The regression equation for Calcutta, which showed the highest value of $R = 0{\cdot}93$, was worked out from the data recorded during the years 1931-33 during the months of November, December and January. Similar sets of observations, one hundred each, were selected for the years 1926-27, 1928-29, 1930-31, the months considered being the same in all the cases. Using the original regression formula (1931-33) the minimum temperatures were calculated in these three cases. The value of R in each case was also calculated by correlating the actual with the calculated minimum temperatures ; these values are given below for comparison.

Sets	Correlation between the 'actual' and the 'calculated' values of minimum temperature
1. (1931-33)	0·93 (R of regression formula)
2. (1926-27)	0·91
3. (1928-29)	0·86
4. (1930-31)	0·88

Similarly in the case of Lahore, one of the stations which showed a minimum value of $R = 0{\cdot}72$, the forecasting formula was tested on three other sets of data. These results are also given below.

Sets	Correlation between the 'actual' and the 'calculated' values of minimum temperature
1. (1927-32, January)	0·72 (R of regression formula)
2. (1917-26, January)	0·70
3. (1916-22, December)	0·78
4. (1922-29, December)	0·66

It may be observed that both in case of Calcutta and Lahore the correlation coefficients have the same order of magnitude as the corresponding value of R, showing that the samples of 100 observations used for calculating the regression equations represent the conditions at each station satisfactorily. The above test constitutes the 'performance test' where the aim is to eliminate the individual bias.

In conclusion we wish to record our thanks to the Director-General of Observatories for the facilities given for conducting this investigation at the Meteorological Office, Poona.

REFERENCES

Normand, C. W. B. (1932). *Quart. J. Roy. Meteor. Soc.* **58**, 3.

Raman, P. K. (1935). *Proc. Ind. Acad. Sci.* **1**, 815.

———— (1936). *Proc. Ind. Acad. Sci.* **4**, 243.

Ramanathan, K. R. and Ramdas, L. A. (1935). *Proc. Ind. Acad. Sci.* **1**, 822.

Ramdas, L. A. (1935). *Current Sci.* **3**, 325.

Ramdas, L. A. and Atmanathan, S. (1932). *Gerl. Beitr. Z. Geopn.* **37**, 116.

Ramdas, L. A.; Sreenivasayya, B. N. and Raman, P. K. (1937). *Proc. Ind. Acad. Sci.* **5**, 45.

Savur, S. R. and Gopal Rao, S. (1932). *Ind. Met. Dept. Sci. Notes* **5**, No. 49.

STUDIES ON THE DISSOCIATION OF *BACILLUS CEREUS* AN ORGANISM ASSOCIATED WITH PLANTS AFFECTED WITH MOSAIC DISEASE

BY

N. V. JOSHI

AND

S. C. DUTT

Imperial Agricultural Research Institute, New Delhi

(Received for publication on 14th June 1937)

(With Plates LXIV—LXVI)

In our search for bacteria associated with mosaic disease in cultivated plants in India we isolated a spore-forming organism from the surface-sterilized tissue of several kinds of infected plants. Among the plants studied mention may be made of tomato (*Lycopersicum esculentum*), *jhingni* (*Luffa acutangula*), *karela* (*Momordica charantia*), *bhindi* (*Hibiscus esculentus*), and tobacco (*Nicotiana Tabacum*) from diseased tissues of all of which the same kind of organism was isolated by following the technique described later on.

The frequency of the association of this organism with the tissues attacked by mosaic as examined by us is given in Table I.

TABLE I

Kind of tissue	Percentage of the number of tissues in which		
	B. cereus alone was found	Cultures other than *B. cereus* were also found	Growth did not occur
Mosaic	70	20	10
Healthy	*Nil*	10	90

Desai [1933] while working in the Bacteriological Laboratory of this Institute was successful in producing necrosis and curling of leaf by inoculating healthy tomato plants and we secured successful results in producing similar mosaic symptoms by inoculating healthy *jhingni* (*Luffa acutangula*) plants with the culture of the organism. More than sixty per cent of the total inoculations made with cultures of this organism in this laboratory were successful in reproducing mosaic symptoms. Mention might be made here of the fact that Desai [1933] inoculated his plants with a dilution of $1 : 10^{17}$ of the juice of the diseased tissues, while our inoculations were made with cultures of the organism obtained from spores after heating and successive transfers, thus destroying any plant juice virus that might have been associated with the first isolation of the organism from the mosaic tissue.

Samples of the soil surrounding the roots of the mosaic-affected, as well as healthy, plants from different localities were plated on four occasions for comparison of the organisms present in them. The samples of soil surrounding healthy plants showed less than one per cent of the total number of colonies, while those of soil in which mosaic-affected plants grew showed more than twenty-five per cent of the total number of colonies to be of an organism exactly resembling the one isolated from mosaic tissues.

We have refrained from giving further details of inoculations and a description of the symptoms produced as well as the actual number of colonies on soil plates because of the inconsistent behaviour of the inoculated cultures in reproducing the disease, and we do not at present propose to establish that the organism isolated by us is the causative agent of the mosaic disease. Other workers [Bewley, 1923 ; Nelson, 1932] have also not obtained conclusive results by inoculation of cultures. These inconclusive results need not, however, prevent us from assuming bacterial origin for the mosaic disease as a working hypothesis and from searching for the probable reasons for failures in reproducing mosaic by inoculations with bacterial cultures.

We might put forward our suggestion in this connection somewhat in the following way. Most of the bacteria hitherto described as causing plant diseases are non-spore-formers. The organism isolated by us is a spore-former and is likely to be regarded as a saprophyte. Recent studies have, however, shown that many bacteria, including spore-formers have different cyclo-stages in their development. It is possible that the organisms, frequently associated with mosaic show changes in life-history and dissociate into several variants and the reason for the non-production of mosaic by inoculation with cultures may lie in the absence of the appropriate cyclo-stages or variants in the culture at the time of inoculation. Before testing the validity of this suggestion, it appeared to us advisable in the first place to study in detail the several changes in the life-history of the organism isolated by us.

Explanation of Plate LXIV

FIG. 1. Agar plate containing the micro-colonies of *G* form of *B. cereus* along with the normal colonies.

FIG. 2. Smooth colonies of *B. cereus* showing the pellucid dots.

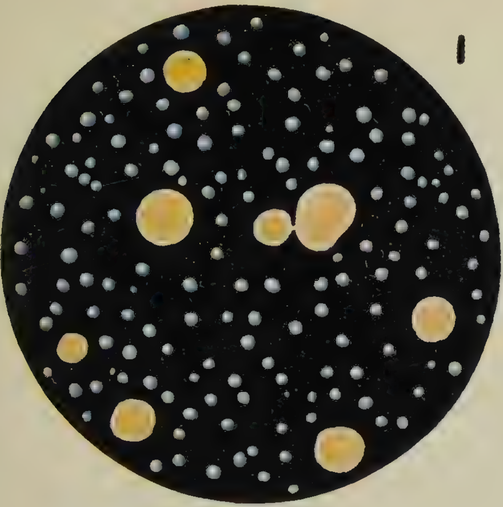
FIG. 3. Agar slant culture of *B. cereus* smooth form.

FIG. 4. Agar slant culture of dissociation form *A* of *B. cereus*.

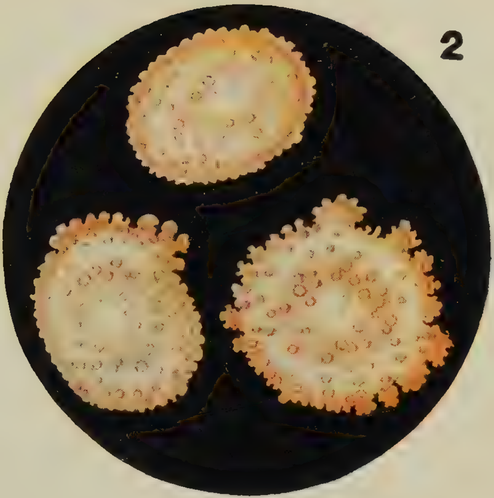
FIG. 5. Agar slant culture of dissociation form *B* of *B. cereus*.

FIG. 6. Agar slant culture of dissociation form *G* of *B. cereus*.

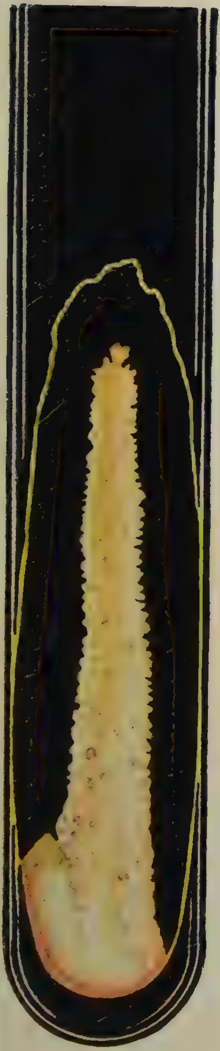
1



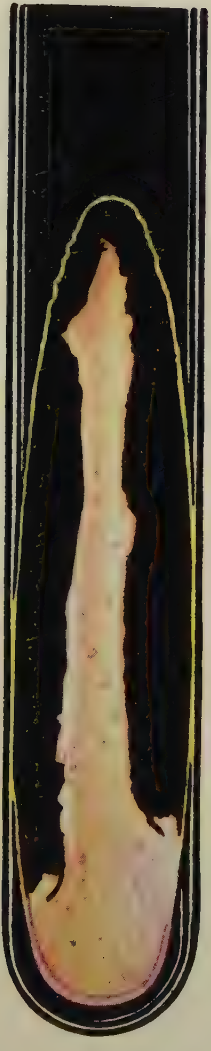
2



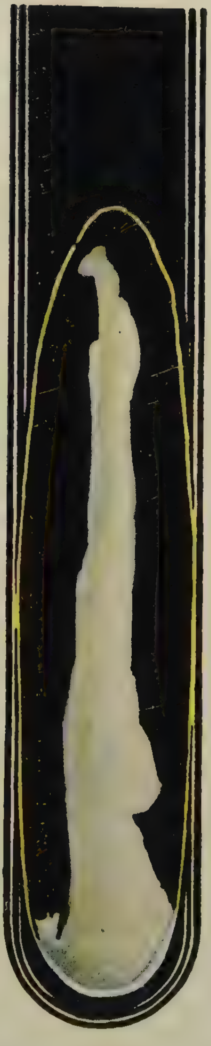
3



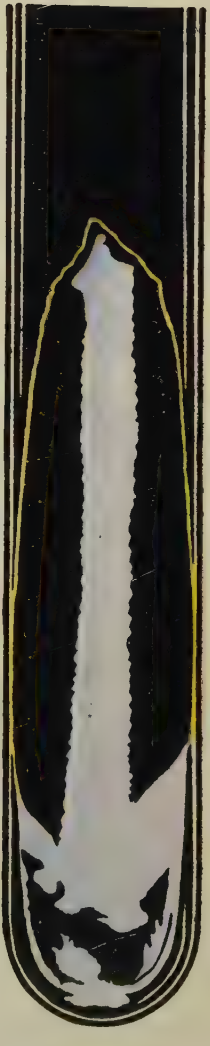
4



5



6



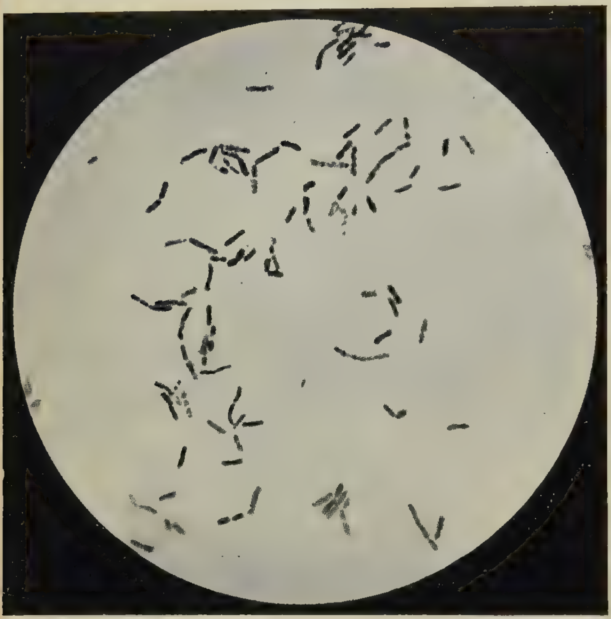


FIG. 1. *B. cereus*, smooth form.

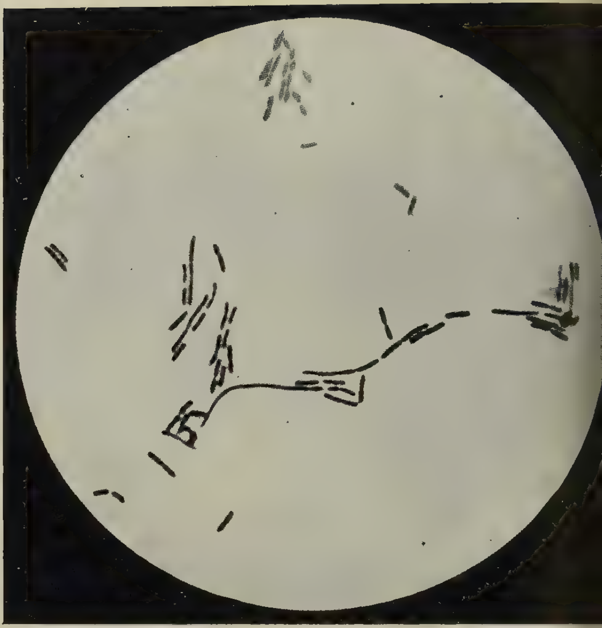


FIG. 2. *B. cereus*, rough form.

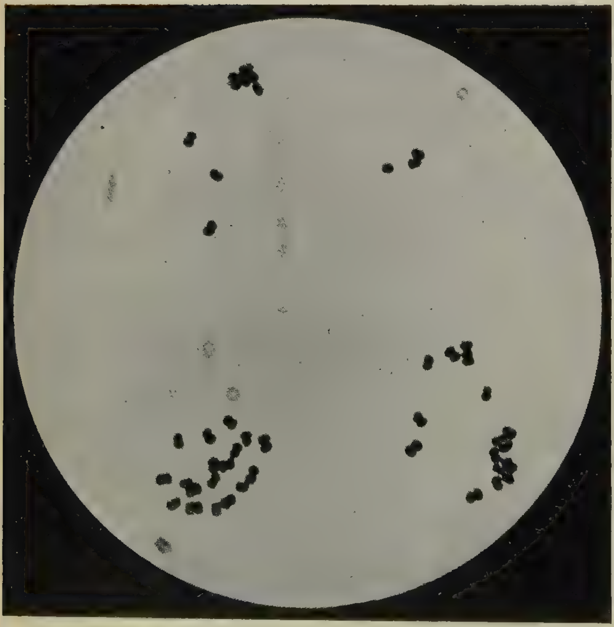


FIG 3. *B. cereus*, dissociation form *A*.

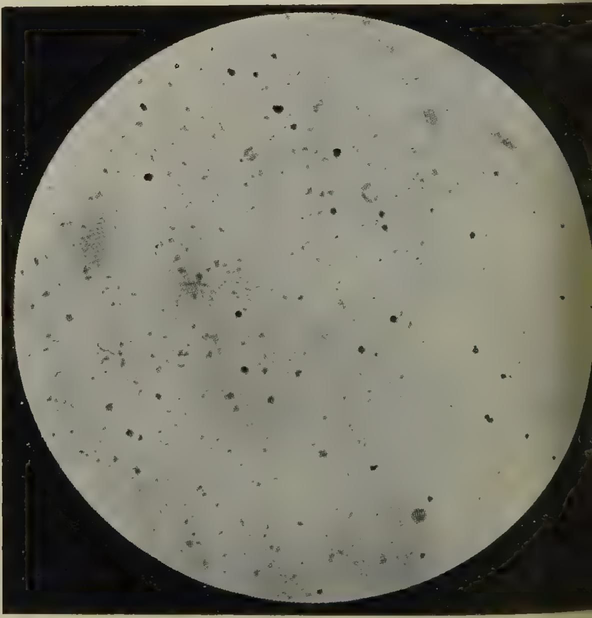


FIG 4. *B. cereus*, dissociation form *B*.

METHOD OF ISOLATION

The technique used for the isolation of the organism from the mosaic-affected plants was a modification of the one used by the senior author in isolating pure cultures from root nodules [Joshi, 1919] and the same as was used by Desai [1933] in his investigation on tomato mosaic. A description of it is given here so as to give readers a correct idea as to the time required for the first appearance of the growth of the organism after the mosaic-affected tissue is put on agar. The petioles of leaves, vines or stems of the diseased plants were cut into pieces about ¾-in. to 1-in. long. These pieces were then put into a sterile test tube and sterilised for ten minutes with a mercuric chloride solution (1 in 1000) by placing the tube in a filtering flask attached to a water pump which produced a vacuum and removed air bubbles. The sterilised pieces were washed several times with sterile water and then lifted with sterile forceps and placed on sterile agar slants and incubated at 30°C. Some pieces were placed in sterile liquid broth. If bacterial growth appeared on agar or in liquid broth within twenty-four to forty-eight hours, the tubes showing such growth were considered as contaminated and were rejected. If the outside surface of the pieces were properly sterilised a bacterial growth generally occurred, after any period between one week to about the end of the third week, at the point of contact of the tissue with the agar slant. In the broth tubes the growth was diffused throughout. The bacterial growth was then plated in different dilutions and on most occasions only one kind of organism could be found on the plates. The organism being a spore-former was further purified by transferring a twenty-four hours' agar growth into a broth tube which was then heated to 100°C. for half an hour. Colonies of the organism were then obtained by plating the emulsion of spores in the heated broth tube, and pure cultures made from single isolated colonies (Plate LXV, fig. 1).

DESCRIPTION OF THE CHARACTERISTICS OF THE ORGANISMS

(*1*) *Morphological.*—Long rods found usually in pairs with granules, size—length 3·6—4μ, breadth 1·2—1·5μ. Motility present, the arrangement of flagella being peritrichic. Gram positive. Endospores present.

(*2*) *Cultural.*—The organism is aerobic and grows profusely on nutrient agar as well as on gelatin. The colonies on agar plates are sometimes round and sometimes irregular with lobate margins. The agar slant growths and the colonies on agar plates show the presence of numerous pellucid dots. The organism liquefies gelatin and grows well in Bouillon with a ring pellicle at the top. It peptonises milk slowly without curdling. Litmus milk is reduced and peptonised.

(3) *Biochemical reactins*—

Reactions with sugars	Gas	Reaction
Glucose	No gas .	Acid after 48 hours. Alkaline after a week.
Saccharose	,, .	Acid.
Lactose	,, .	No change.
Reaction with nitrate	,, .	No change.
Methyl red reaction		Alkaline.
Indol formation		No indol formed in peptone water.

From the above morphological, cultural and biochemical tests the organism is tentatively identified as *B. cereus* (Frankland). It may be observed that the above description differs from the description of *B. cereus* as given by Bergey [1930] and the description of the organism of tomato mosaic as given by Desai [1933] in two particulars, *viz.* the breadth of cells and the reaction in glucose peptone. The difference in breadth is not important because it is well known that under conditions of cultivation in the laboratory, organisms may vary in their size, and moreover the breadth 1·2 to 1·5μ observed by us for our organism agrees very well with 1·3μ which is given by Chester [1903] in his description of *B. cereus.* The difference in reaction in glucose peptone is due to the difference in the periods of incubation after which the observations were made. Bergey records the reaction at the end of forty-eight hours and gives it as acid. We also noted variable production of acid in glucose peptone in the first few days, but have recorded the final reaction after a week's growth as alkaline. Thus the differences noted above do not appear to be important enough for coining a new name for our organism.

In studying the description of the growth of *B. cereus* on agar, we find a mention of the pellucid dots made by Bergey *et al.* [1930], but there had been no attempt at any explanation of the peculiar appearance of pellucid dots in the agar growth of *B. cereus* until Stearn and Stearn [1933] carried out experiments to test whether the cultures of *B. cereus* are associated with a lytic principle and concluded that the organism is associated with a lytic principle or bacteriophage, which when producing lysis of the bacterial cells causes the pellucid dots to appear in the growth of the organism on agar. The pellucid dots are thus considered by these investigators to be the lysed spots caused by a bacteriophage, which is associated with the organism. Desai [1933] working in this laboratory, examined the cultures of the organism isolated by him from tomato mosaic for a bacteriophage in the pellucid dots or the so-called 'lysed spots' of Stearn and Stearn [1933]. Having failed to isolate any lytic principle or phage from the pellucid dots, Desai considered that the pellucid dots are places where the culture exists 'in virus form', as distinct from the surrounding growth consisting of the normal form of the organism. According to this view, the cause of the occurrence of the pellucid dots may be explained to be the simultaneous growth of the normal and the virus form of the organism. Desai [1933] assumed ' the virus form ' to be a filterable cyclo-stage in the life-history of his tomato organism which closely resembles *B. cereus* ; he has not, however, attempted to give any definite proof for this assumption of his.

Since the suggestion has been put forward by Bewley [1931] that the principle causing the tomato mosaic is of the bacteriophage type, it is considered necessary to examine critically the difference of opinion, as noted above, between Stearn and Stearn [1933] and Desai [1933] about the existence of a lytic principle or bacteriophage in *B. cereus* and also to seek an explanation of the pellucid dots in the cultures of *B. cereus* isolated from the mosaic-affected plants.

Attempts at isolation of the bacteriolytic principle from the cultures of B. cereus

As several methods are in use for the separation of the organism from its associated bacteriolytic principle or bacteriophage, a description of the methods used by us to separate the organism and the supposed bacteriolytic agent from each other is included here. It will give an idea of the actual experimental data obtained by us and enable readers to judge whether the conclusions drawn by us are justified or not.

(*i*) *Increasing the virulence of the supposed bacteriolytic principle associated with* B. cereus.—Transfers were made from the agar cultures of *B. cereus* with pellucid dots into peptone broth of *p*H 7·8-8·0 to isolate the phage and increase its virulence by filtrations of the several successive broth cultures. One of the tubes of broth cultures was filtered through a L_3 Chamberland filter candle at a pressure of —200 mm. Any bacteriophage associated with the culture would naturally pass into the filtrate. This filtrate through the candle is hereafter called the 'first phage filtrate'. Into each of the two sterile broth tubes one c. c. of a fresh culture (18-24 hours old) of *B. cereus* was inoculated and to one of them was added aseptically one c. c. of the ' first phage filtrate ' and the tubes were incubated at 30°C. After twenty-four hours' incubation, the difference in turbidity, if any, was noted down and the tube containing the phage was filtered through a L_3 candle, the filtrate being called ' the second phage filtrate '. Two fresh broth tubes were inoculated again with one c. c. each of a twenty-four hours culture and to one of them 0·5 c. c. of ' the second phage filtrate ' was added and the tubes incubated as before at 30°C. continuing in this way about fifteen transfers and filtrations were carried out to increase the virulence of the phage. During the whole course of these filtrations no appreciable clearing was noticeable in any of the tubes, to which 0·5 c.c. of the successive phage filtrate was added, the turbudity in the phage-added tubes being equal to that of the control tubes.

(*ii*) It might be argued that on account of the constant association of the bacteriophage with the organism whatever alterations and adaptations between the organism and the phage were to take place must have already taken place and hence 'the phage filtrates' would not be able to show any visible difference in the turbidity of the liquid culture. It was, therefore, decided to see the effect of 'the phage filtrate' obtained by the process, as outlined above, on the continuous layer of growth of *B. cereus* on solid media by 'the short-cut method' of ascertaining the presence of a bacteriophage described by Asheshov [1933] in his studies on cholera bacteriophage. A streak of the twenty-four hour culture of *B. cereus* was made on an agar plate with a spreader loop (flattened loop to hold a large amount of fluid) and when the culture had dried on the agar it was touched at different points with a small loopful of the phage filtrate and the plate incubated at 30°C. In the agar growth of *B. cereus* no typical clearings could be observed, where the loops of the 'fifteenth phage filtrate' were added. An idea of the kind of the typical clearings that should have made their appearance on the agar growth of *B. cereus*, had any lytic principle been present in the 'fifteenth phage filtrate', can be obtained from Plate LXVI, fig. 3 where the action of a bacteriophage of the legume organism is shown on the cultures of the berseem nodule organism.

(*iii*) To obtain the organism in ultra-pure state it was repeatedly plated on ordinary agar of *p*H 7·8, but even after twenty platings, the pellucid dots made their appearance.

(*iv*) After several repeated transfers and platings on bile salt media, the colonies which developed after each successive plating, as well as after the final plating, exhibited the usual pellucid dots.

(*v*) Media with different hydrogen-ion concentrations of *p*H 6 to *p*H 8 and also media with different sugars—glucose, sucrose and lactose—added to them in varying concentrations were used to separate the organism from its phage, supposed to be associated with and expected to be present in the pellucid dots, without any success.

(*vi*) The organism was grown from spores by heating it in broth culture at 100°C. for half an hour, which would have the effect of killing the vegetative cells as well as any bacteriophage associated with it. The first culture on agar obtained from it was transferred to broth and again subjected to heat and the process was repeated successively ten times, and it was found practically impossible to get an ultra-pure culture free from pellucid dots.

(*vii*) As the media of different hydrogen-ion concentrations (*p*H 6 to *p*H 8) used to grow the organism had failed to give a culture free from the pellucid dots resembling the plaque-like areas lysed by a bacteriophage, it was suggested that media, more widely varying in their hydrogen-ion concentration, might be used to grow the organism, to find out the limits of acidity and alkalinity tolerated by the organism and the phage respectively. If the limits of acid or alkali tolerance by

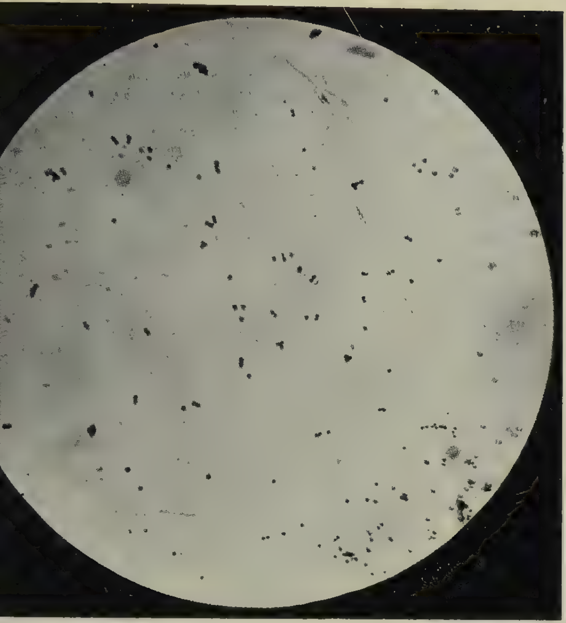


FIG. 1. *B. cereus* dissociation form *G.*

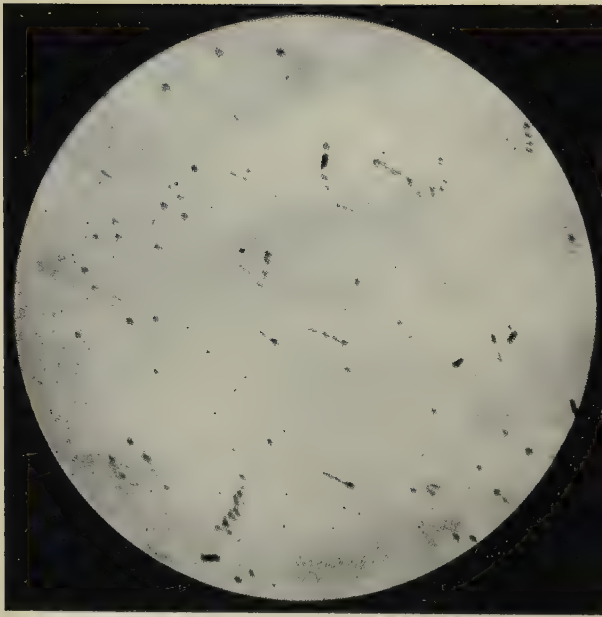
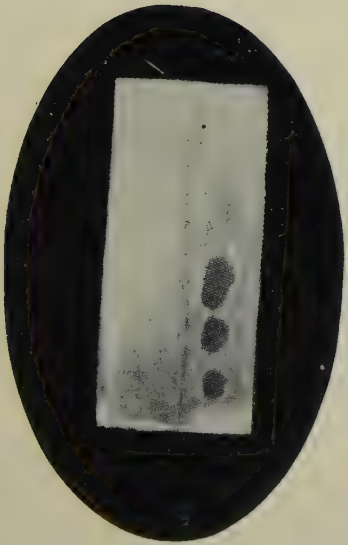


FIG. 2. Showing the different-sized organisms, son granules with flagella, derived from the secondary growt in the filtrate of *B. cereus* cultures 1-8 days old.



Showing the typical clearings by berseem nodule on berseem nodule organisms on agar plate.

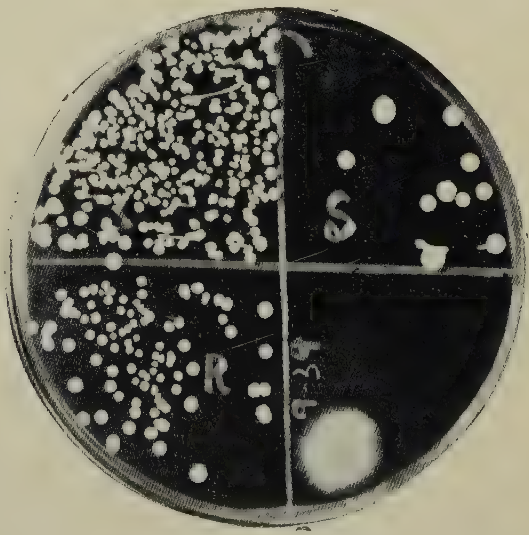


FIG. 4. Poured agar plate divided into four quadrants where organisms were plated.

the organism and the phage did not differ they could never be separated from each other; but if the limits of tolerance by the organism and the phage were found to differ, it would be merely necessary to grow the organism in broths of the highest acidity or alkalinity tolerated by it, so that the bacteriophage being unable to survive in the highly acid or alkaline broths would not grow in them and subsequent sub-cultures from such broths would be pure cultures of the organism freed from the bacteriophage. For this purpose several tubes containing ten c. c. of sterilized broth were taken and divided into two series. In one series 0·1 c. c., 0·2 c. c., 0·3 c. c., 0·4 c. c., and 0·5 c. c. of $N/2$ caustic soda were added respectively to the first five tubes and one tube was kept without any addition as control (with a *p*H 8). Similarly by the addition of 0·1 c. c., 0·2 c. c., 0·3 c. c., 0·4 c. c. and 0·5 c. c. $N/2$ hydrochloric acid a second series of tubes of broth of varying acidity was prepared. All the tubes were inoculated with the culture of *B. cereus*. Growth was examined in both the series of tubes after twenty-four hours' incubation. It was found to be restricted to tubes which had received lower quantities of alkali or acid. From the tubes in which growth had taken place the tube with the greatest concentration of alkali in the first series was then used for inoculating 0·5 c. c. of the culture from it into the tube with next higher concentration of alkali, and when growth took place in this new tube, 0·5 c. c. of the culture from this new tube was transferred to the tube with the next higher concentration of alkali, and so on. Similar transfers were made till the limit of toleration of the alkali by the culture was reached. By making the culture in this manner adapt itself to the highest alkali concentration possible, a strain tolerant of high amounts of alkali could be obtained. By similar treatment in the acid series we could get a strain of the culture adapted to the highest acid concentration possible.

The underlying assumption in obtaining such strains is that while the bacteria are adapting themselves to the higher alkali or acid concentration, the associated lytic principle or bacteriophage, if present, should disappear, as it cannot adapt itself to the drastic changes in the hydrogen-ion concentration with the same rapidity as that of the organism.

In this process of transfers to higher concentration of alkali and acid, the cultures of *B. cereus* were ultimately found to grow in the tubes to which 0·4 c. c. of $N/2$ caustic soda was added in the first or the alkali series and in the tubes to which 0·2 c. c. of $N/2$ hydrochloric acid was added in the second or acid series.

The growths from both these tubes were then plated on ordinary agar and it was found that the colonies that grew on agar from the acid tube culture showed the pellucid dots, while the colonies from the alkali tube were of two types: some were of the usual pellucid dot type and the others were dew-drop like in appearance. The organisms from the dew-drop like colonies, when examined under the microscope, appeared like small cocci instead of being rod-shaped like the organisms from the colonies with pellucid dots, As a result of all the foregoing experiments, we came to the conclusion that the pellucid dots observed in the growth of *B. cereus* on agar are not the result of lysis caused by any bacteriophage or bacteriolytic principle associated with the organism. Incidentally this conclusion disposes of the suggestion that a bacteriophage of the organism under study may be the cause of the mosaic in the different plants. We were not in a position to use Bordet's anti-serum method for separation of the associated bacteriophage from a mixed bacterial culture, and hence we gave up further pursuit of the separation of the bacteriolytic principle alleged to be associated with *B. cereus* in culture. Moreover the appearance of two types of colonies observed after plating 'the highest concentration of alkali tube' culture of *B. cereus* on agar in the last experiment suggested that a plausible explanation of the pellucid dots in the cultures of *B. cereus* may lie in a different direction, as it is possible to imagine that the closely associated growth of colonies of two variants of *B. cereus*, arising out of a natural dissociation in agar cultures, may cause the deceptive appearance of lysis in the form of pellucid dots.

Microbic dissociation.—Having arrived at the conclusion that no bacteriolytic principle is concerned in the production of pellucid dots in the cultures of *B. cereus* we directed our attention to a study of bacterial dissociation. A brief reference to the important observations made by previous investigators on general microbic dissociation, before describing our experiments with cultures of *B. cereus*, may, we hope, not be considered out of place. In the early period, before the method of obtaining pure cultures was discovered, the prevalent idea was that any bacterium was capable of transmutation or of assuming various different shapes or cell-forms in its complex life-cycle. This view had been replaced by the monomorphistic theory of Koch who discovered the method of cultivating a bacterium in pure cultures and showed that the transmutation spoken of by previous workers was due to their working with impure cultures consisting of bacteria of different cell forms and that under constant cultural conditions a pure culture of a bacterium was incapable of changing its form. This monomorphistic theory ruled for a number of years till a small group of bacteriologists [Almquist, 1911 ; 1922 ; Hort, 1917 ; Löhnis, 1916 ; Mellon, 1919, 1926, 1927 and Enderlein, 1925] made their initial contributions on the pleomorphistic theory concerning the nature of the morphological variations of bacteria. Of these workers Löhnis [1916, 1921], working with a well-known soil organism *Azotobacter*, noticed a great number of varieties of cell forms in the life-cycle of this organism. His investigation into the life-cycle of *Azotobacter* and other organisms led to the discovery that from cultures of many other organisms different growth types might be developed. He has described seven such types, *e.g.* coccoid forms, fungoid cell, small and large, sporulating and non-sporulating cells and dwarfed cell types. Löhnis [1916] also noticed the filterable forms of *Azotobacter* and other organisms.

Jones [1920] confirmed the data of Löhnis so far as the pleomorphism of *Azotobacter* cells was concerned.

Mellon [1919, 1926, 1927] in a series of papers on microbic heredity has described morphological variations in some of the pathogenic bacteria and has shown that bacteria pass through a more or less complex cycle in which various types — rods, spheres, branching forms, etc.,—appear and that any of these types may be stabilized. He also pointed out the possibilities of bacteria passing through a phase in their life-cycle in which they are invisible and capable of passing through porcelain filter.

Several investigators have shown that the addition of different chemicals, such as sodium chloride and lithium chloride may have an important effect on the dissociation of bacterial cultures into filterable forms [for a summary see Hadley, 1927].

Hadley and others [1927, 1931] have also studied the dissociative variations in the life-cycle of Shiga dysentery bacillus comprising the method of production, the cultural behaviour, the morphology and filterability. They produced the filterable virus-like stage as well as another which they call ' the *G* type culture ' of this organism by forcing the dissociative reaction on the mother cultures of *S* and *R* type, and from the filtrates (Berkfeld N & W candles) of *S* and *R* cultures they were successful in recovering this *G* type culture which differed culturally, morphologically, biochemically, serologically and immunologically from the normal forms of the Shiga bacillus. They have suggested to the future workers " to incorporate the point of view of microbic dissociation in the study of certain so-called virus diseases ".

In our studies on dissociation of *B. cereus* we generally followed the technique of serial transfers in broth, with and without lithium chloride, as adopted by Hadley [1931]. For our study we have used broth of the following composition :—

Peptone	5 grms.
Marmite	2 grms.
Sodium chloride	5 grms.
Water	1000 grms.
Adjusted to *p*H	7·8 to 8·0

Lithium chloride broth contained 0·25 per cent lithium chloride in addition to the ingredients of the ordinary broth mentioned above.

The culture of *B. cereus* as obtained from spores by heating the culture to 100°C. for half an hour for ten successive generations was used for this study and was inoculated in a pair of ten c. c. broth tubes, one with and the other without lithium chloride and incubated at 30°C. After twenty-four hours' growth one standard 3 mm. loop of the culture (about 0·025 c. c.) was transferred in each case to another similar pair of tubes, the organisms in the first pair were plated after appropriate dilution (1 in 400). Instead of using one plate and a glass rod for spreading the growth according to Hadley [1931] we found it convenient to use the method recommended by Asheshov [1933]. This consists in dividing a poured agar plate into four quadrants by making two glass pencil lines through the centre of the Petri dish, 12 cm. diameter, at right angles to each other (Plate LXVI, fig. 4). One loopful of culture after dilution from one of the pair of tubes was smeared with the loop on one quadrant of the dish and from the other tube on a second quadrant and the dish incubated at 30°C. The other two quadrants in the same Petri dish could be used again next day if necessary. This dilution and the method of plating gave well-isolated colonies of the organism which appeared in the dish after twenty-four hours. After twenty-four hours a third pair of broth tubes were similarly inoculated from the second pair and the organisms plated from the second pair after dilution. This process of transfers and platings was carried out till twenty successive plates were obtained. The types of colonies were examined and stained preparations on glass slides were made from each type for examination under the microscope and all the different types of colonies encountered in the course of this study were cultivated on agar and maintained in a pure state without contamination for studying their cultural characteristics and biochemical reactions. This experiment of successive transfers and platings was repeated about ten times, so that no forms may be missed and to ensure confirmation of the fact that the forms isolated by us were really derived from dissociation of the cultures.

The following forms were obtained and identified as variants from the dissociation of cultures of *B. cereus* studied :—

I (*A*) *Swollen rods,* resembling cocci having a diameter of 1·8 to 2·2 μ. This form is also seen in agar growth twenty-four hours old. It forms small reddish tinged colonies on agar. This is obtained less frequently than the small coccal form or coccal rod form mentioned later. Once separated from the mother organism it does not revert to the original mother form. It was Gram positive and it did not form spores (Plate LXV, fig. 3).

II (*B*). *Coccal rod.*—Short rod-shaped organism having a length of 0·50 to 0·75 μ and breadth 0·3 to 0·40 μ. It forms small dew-drop like colonies having a yellow tinge on agar plate. It is motile having a single polar flagellum, and is Gram positive. It does not form spores. This is a stable form and attempts to revert it to the original form were not successful (Plate LXV, fig. 4).

III (*C*) *Cocci.*—Small coccal form having a diameter of 0·4 to 0·7 μ. This is similar to the *G* type of Shiga bacillus first described by Hadley [1931], and will be referred to as *G* hereafter in this paper. Its colonies on agar appear as dew drops having bluish appearance by transmitted light and whitish by reflected light. This form is obtained generally in the 3rd to 5th plates from the lithium chloride broth series and in the 7th to 10th plates in the ordinary broth series. This is also a very stable form and our attempts to revert it to the original form by continuous transplants on agar to agar or broth to agar were not successful, although Hadley and others report that they were successful to cause the *G* form to revert to the original cell type in the case of Shiga dysentery bacillus (Plate LXVI, fig 1).

IV. Non-sporulating non-motile 'R' variant.—Very recently another variant of *B. cereus* was found by us while plating an old broth culture of the organism. It is a non-sporulating rod-shaped organism, similar in appearance to the usual sporulating organism, in its vegetative stage, and forms translucent colonies on agar plates having crinkled appearance. The peculiarity of the colonies of this variant is that they are similar to those of the normal sporulating type but without the pellucid dots (Plate LXV, fig. 2). This new variant was not obtained by Stearn and Stearn [1933] nor by Desai [1933], although these investigators were searching for it inasmuch as they were trying to obtain colonies of *B. cereus* without the pellucid dots.

When an old agar slant culture of *B. cereus* is examined under the microscope most of the cells appear to have formed spores, but there are some which still persist in their vegetative forms. The ratio of non-sporulating cells to the sporulating cells is very low in old agar slant cultures but of the total number of cells in old broth cultures the percentage of non-sporulating cells present is very high. That is the reason why on plating an agar culture of *B. cereus* colonies of the sporulating cells mostly develop, while on plating an old broth culture the colonies of the non-sporulating cells are chiefly formed. This peculiar difference in the nature of variants during the spontaneous dissociation of *B. cereus* cultures on solid and liquid media is sufficient to explain why the previous investigators did not obtain colonies of the non-sporulating form of this organism, *i.e.* colonies without pellucid dots. From an examination of the nature of the colonies we consider this new variant which consists only of non-sporulating cells to be the *R* (rough) form and the normal culture, which has sporulating cells, to be the *S* (smooth) form of *B. cereus.*

An examination of the table of cultural characteristics appearing later on will show that this *R* (rough) form is similar to the smooth form except that it is non-sporulating and non-motile. Perhaps, owing to its being cultivated in broth for a long time, the cells may have lost their power of spore-formation and locomotion.

Dissociation of R *(rough) form of* B. cereus.—Following the same technique of serial transfers and platings as was used with the smooth form it has been possible to obtain dissociative forms from the *R* form of *B. cereus.* Both coccal rod (*B*) and coccal forms (*G*) variants have been isolated as colonies at the 9th or 10th transfers both in plain and in lithium chloride broth, but big swollen coccal variant (*A*) was not observed. As the '*R*' form was obtained much later in the course of our investigation, attempts to obtain the variants of the *R* (rough) form could so far be made on five occasions only. The coccal rod and coccal forms obtained from the *R* form were similar to those obtained from the smooth form, morphologically as well as culturally and biochemically.

Table II describing the morphological and cultural characteristics and biochemical or physiological reactions of the several variants is given below.

TABLE II

Showing the characteristics of B. cereus *and its different forms*

	Morphological				
	Form and arrangement	Size in μ	Gram staining reaction	Motility	Spore formation
B. cereus smooth	Long stout rods, usually in pairs.	3·6—4·0 × 1·2—1·5	Positive	Motile with peritrichic flagella	Spores formed
B. cereus rough	Long stout rods, usually in pairs and long chains	2·2—3·5 × 1·0—1·2	,,	*Nil*	Spores not formed
B. cereus : Dissociation Form *A*	Stout cocci in pairs	1·8—2·2	,,	*Nil*	,,
B. cereus : Dissociation Form *B*	Very short rods	0·5—0·75 × 0·3—0·4	,,	Motile single flagellum	,,
B. cereus : Dissociation Form *G*	Micro cocci	0·4—0·7	,,	*Nil*	,,

TABLE II—*contd.*

Showing the characteristics of B. cereus *and its different forms*

	Cultural					
	Gelatine stab	Agar slope	Bouillon growth	Potato growth	Milk	Litmus milk
B. cereus smooth	Liquefies gelatine	Yellowish brown growth with pellucid dots	+ + + with sediment and ring pellicle	+ + + diastase	Peptonised without curdling	Reduced peptonised
B. cereus rough	Liquefies gelatine	Yellowish brown without pellucid dots	+ + Hazy, sediment settling N. P.	+ + +	Peptonised without curdling	Reduced peptonised
B. cereus : Dissociation Form *A*	Slight or no growth	Pale red growth	N. P. + Hazy	Very slight or no growth	No curdling. No peptonisation	No reduction. No peptonisation
B. cereus : Dissociation Form *B*	”	Pale yellow growth	N. P. + Hazy	”	No curdling. No peptonisation	No reduction. No peptonisation
B. cereus : Dissociation Form *G*	”	Bluish white	N. P. + Hazy	”	No curdling. No peptonisation	No reduction. No peptonisation

+ + + Heavy growth.
+ + Medium growth.
+ Slight growth.

N. P. No pellicle.
N. C. No change.
N. T. No turbidity.

TABLE II—*concld.*

Showing the characteristics of B. cereus *and its different forms*

	Biochemical																
		Glucose			Saccharose			Lactose			Glycerol			Nitrate broth		
	Indol	Gas	Reaction	Growth	Gas	Reaction	Growth	Gas	Reaction	Growth	Gas	Reaction	Growth	Gas	NO_2	NH_3
B. cereus smooth . .	—	—	Acid to alkali	N. P. Hazy	—	Acid .	N. P. Hazy	—	N. C.	N. P. Hazy	—	N. C.	N. P. Hazy	—	—	—
B. cereus rough . .	—	—	Acid to alkali	N. P. Hazy	—	Acid .	N. P. Hazy	—	N. C.	N. P. Hazy	—	N. C.	N. P. N. T.	—	—	—
B. cereus : Dissociation Form *A*	—	—	Alkali .	N. P. N. T.	—	N. C.	N. P. N. T.	—	N. C.	N. P. N. T.	—	N. C.	N. P. N. T.	—	Present	—
B. cereus : Dissociation Form *B*	—	—	Alkali .	N. P. N. T.	—	N. C.	N. P. N. T.	—	N. C.	N. P. N. T.	—	N. C.	N. P. N. T.	—	Present	
B. cereus : Dissociation Form *G*	—	—	Alkali .	N. P. Hazy	—	Acid .	N. P. Hazy	—	N. C.	N. P. Hazy	—	N. C.	N. P. Hazy		Present	

— Absent.

N. P. No pellicle.
N. C. No change.
N. T. No turbidity.

V. Filterable forms of B. cereus.—In the course of the experiments involving filtration of the cultures of *B. cereus* it was observed that on many occasions bacterial growth occurred in the filtrates which were filtered through L_3 Chamberland filter candles, when the filtrates remained at the laboratory temperatures for a few days. Our first impulse was to consider these secondary bacterial growths to be caused by contamination from outside because the bacterial growth did not occur in all the filtrates. The repeated occurrence of the same kind of growth, however, in the majority of the filtrates and the exact resemblance of these secondary bacterial growths in the filtrates to the original culture, both as regards morphological and cultural characteristics, led us to carefully examine the question as to whether this occurrence of secondary growths in the filtrates is not connected with what may be termed the 'filterable phase' of *B. cereus*. Several investigators including Löhnis, Mellon and Hadley have already reported that certain bacteria in their life-cycle pass through a stage in which their cells become so minute as to be invisible under the highest power of the microscope. In this stage the ultra-microscopic cells are so minute that they can pass through the pores of the porcelain filter candles. If the *B. cereus* cultures at the time of filtration contain such minute cells, then they may be expected to pass through L_3 Chamberland candle filters and give rise to the secondary growths in the filtrates by regenerating themselves to the original cell form, after a certain interval provided the filtrate is kept at a suitable temperature and contains sufficient food and energy material.

EXPERIMENTAL

To test the correctness of this possible explanation of the appearance of secondary growths in the filtrates, a number of tubes containing 10 c. c. broth of *p*H 7·8 were inoculated with *B. cereus* and incubated at 30°C. These tubes were filtered through L_3 Chamberland candle filters under a pressure of — 200 mm. Each one of the tubes was incubated for a different period of time before filtration. The filtrates obtained were incubated at 30°C. and kept under observation to see whether any visible growth occurred and note the time of its occurrence. The results are given in Table III.

TABLE III

Showing the number of days taken for the appearance of visible growths in the filtrates of cultures of B. cereus *various days' old*

Organism	Number of days incubated before filtration is performed	Interval between the time of filtration and the time of appearance of secondary growth or examination of filtrates			
		Number of days	1 month	2 months	3 months
1. *B. cereus* .	1	2			
2. ,, .	2	6			
	2	2			
3. ,, .	3	2			
4. ,, .	4	1			
	4	1			
	4	3			
5. ,, .	5	1			
6. ,, .	6	1			
7. ,, .	8	2			
8. ,, .	9	No growth	No growth	No growth	No growth
9. ,, .	12	,,	,,	,,	,,
10. ,, .	12	,,	,,	,,	,,
11. ,, .	13	,,	,,	,,	,,
12. ,, .	35	,,	,,	,,	,,
13. ,, .	35	,,	,,	,,	,,

Before discussing the results in Table III, it may be mentioned that the porosity of all the L_3 Chamberland candle filters was tested before using them for filtration by subjecting them to a positive pressure of eighteen to twenty lbs. with a force pump and only such candle filters as did not show air bubbles passing through below eighteen lbs. pressure were considered to be free from the defects of having big-sized pores or leaks or cracks and selected for filtration in our experiments. Candles showing air bubbles only after 22 lbs. pressure were burned and tested again.

This selection of faultless filter candles and the fact that filtrates of nine days' to thirty-five days' old cultures did not show secondary growths are, in our opinion, sufficient proofs of the correctness of the conclusions drawn from this experiment.

The two facts that are obvious in the above table are that (1) the visible growths always occurred in the filtrates of *B. cereus* cultures which were from one to eight days' old and the period of time required for these filtrates to show visible growths varied from one to six days and that (2) no visible growths occurred in the filtrates of cultures more than eight days' old at the time of filtration, although the filtrates were kept under observation for three months.

On examination of slides prepared from the secondary growths in the filtrates (Plate LXVI, fig. 2) one could observe different-sized organisms, from minute dots up to the size of the organisms in the original culture. We conclude, therefore, that cultures of *B. cereus* from one to eight days' old contain such minute cells as will pass through L_3 Chamberland filter candles, and that these minute or filterable cells, passing through the L_3 Chamberland filter candle into the filtrate if incubated at a suitable temperature revert to the original forms by gradual evolution and ultimately develop into a growth visible to the naked eye. These filterable cells represent the filterable stage in the life-cycle of *B. cereus.*

The fact that the filtrates of *B. cereus* cultures more than eight days' old failed to show any visible growth after a period of long incubation may mean either that the filterable cells are not present in the filtrate or that the filterable cells, though present, do not easily revert to the original culture. The assumption underlying the latter alternative is that the filterable cells which are present in the filtrates of cultures possess the distinctive property of reverting to the original cell forms, but when the cultures attain a certain age limit (with *B. cereus* cultures this limit appears to be eight days) the filtrate does not contain sufficient food material for regeneration into visible growth. In such a contingency the filterable cells become stabilised as a filterable strain on account of the continued dissociation during the period of eight days' growth to such a degree that they cannot revert to the original form, except under some stimulus. The test of the correctness of this assumption lies in supplying an appropriate food or stimulus and observing whether there is any reversion to some variant of the original culture. Such a test of supplying a stimulus was applied by us.

It has already been demonstrated by Hauduroy [1929] that such apparently sterile broth culture filtrates, in which no visible growths occurred under the natural conditions, developed visible growths on agar or solid media. Hauduroy's method consisted in the transfer of an inoculum of five to ten drops of the filtrate on a sterile agar plate and after two or three days' incubation, washing the plate with ten to fifteen drops of sterilized water and again seeding a second plate with five to ten drops of the washing and continuing these serial plate transfers at intervals, till visible growth occurred. We followed Hauduroy's technique except that we used sterile agar slants for the serial transfers of the inoculum from the filtrates and washings, as there was a great possibility of contamination during the inoculation and washing of agar plates under the climatic conditions prevalent here. For this purpose some of the filtrates of cultures of *B. cereus* which were not showing any visible growth were selected and 0·5 c. c. of each of the filtrate was transferred to separate sterile agar slants. After three or four days' incubation of the inoculated tubes in slanting position, to allow the filtrates to be in direct touch with agar slope, 0·5 c. c. of sterile broth was added to each of the tubes and the agar slope was washed with the amount. From these washings 0·5 c. c. was transferred from each tube to a fresh agar slant. Continuing in this way in the third or fourth transfer we obtained on almost all occasions, visible growth which at first had the appearance of a thin film on agar but which gradually developed into a thick growth resembling that of *B. cereus.* On examining slides from this thin film under the microscope it was found to contain organisms of different sizes, as was previously noticed in the secondary growths from the filtrates of cultures which were less than one week old, showing that the film-like growth must have developed from the filterable cells in the filtrates. From these observations we conclude that the filterable forms of *B. cereus* are present in cultures, which are more than one week old, but are so stabilized that they do not easily revert to the original culture forms even if the incubation period is extended to three months.

ORIGIN OF PELLUCID DOTS

After isolating the several variants in stabilised forms we made attempts to produce pellucid dots by growing the different variants together. In the first instance, before the isolation of the *R* variant, we had tried different combinations of the variants. It was of course fruitless to include cultures of the *S* form, the usual spore forming culture, as it could always produce pellucid dots by itself. None of these combinations of associative growths of the different variants showed the pellucid dots. The isolation of the new variant of *B. cereus* from old broth cultures provided a fresh opportunity to test the production of pellucid dots on the smooth growth of this *R* form of *B. cereus* by allowing the other variants to grow in association with it.

EXPERIMENTAL

Broth cultures of *R* form and other variants (*A*), (*B*), (*G*) described above and the filterable phase were made. After incubation for twenty-four hours six streaks of the culture of the *R* form were made on a poured agar plate, with a spreader loop. The first streak was kept as control. On the second streak of the *R* form a streak of the culture from dissociation form the big cocci variant (*A*) was made. With another spreader loop on the third streak of *R* form a streak of the culture from the dissociation form the coccal rod variant (*B*) was made. On the fourth streak a streak of the culture of the dissociation form the cocci form variant (*G*) was made. On the fifth streak, a streak of the filterable form culture was made and on the sixth streak, streaks of the cultures of all the variants were made ; and the plate was then incubated for two to three days. When the plate was examined, typical pellucid dots had appeared on the third, fourth and sixth streaks only, *i.e.* where the rough form was associated with coccal rod variant (*B*) and coccal form variant (*G*) and where all the three other variants were growing. The appearance of pellucid dots was a little more pronounced with the coccal rod form variant (*B*) than with the other variants. Where all the variants were growing together including the (*B*) and (*G*) variants it is but natural to expect the pellucid dots. The absence of the appearance of pellucid dots with other variants shows that they are not responsible for the pellucid dots. There may be a possibility that the filterable forms may show the production of pellucid dots after a longer time of incubation, but in the usual cultures from the *S* form the pellucid dots appear within a period of three to four days' incubation and hence only those variants which produce the pellucid dots in association with the '*R* form' within the period of three to four days' incubation can be considered as responsible for producing that result in the usual cultures of the *S* form. The appearance of pellucid dots in the cultures of the spore-forming variant of *B. cereus* can now be easily explained, because this '*S* form' variant is found to dissociate into all the other variants all of which may, therefore, be present in its culture on agar. It has also been shown above that when the *R* form grows in association with either the coccal rod variant (*B*) or the coccal variant (*G*) pellucid dots are produced. The pellucid dots in the cultures then are the spots where *R* form has come in close association with *B* or *G* type variants.

This experiment was repeated four times but on all occasions only the two variants mentioned above produced the pellucid dots in association with the *R* form.

SUMMARY

An organism was found to be practically constantly associated with different kinds of plants, *e.g.* tomato, tobacco, *bhindi*, etc., which were suffering from the mosaic disease. The technique of isolation consists in (*a*) sterilising the outside of the cut pieces of stems and petioles of leaves by 1 : 1000 mercuric chloride solution under reduced pressure, (*b*) washing the sterilised pieces with sterile water and placing them on sterile agar slants or in Bouillon tubes and (*c*) selecting only such growth as occurred after an inbucation period of seven to ten days, sometimes extending to twenty-three days. Any tube in which growth occurs before seven days' incubation is rejected as being contaminated.

Inoculation with cultures obtained in this way into healthy plants gave positive results on many but negative ones on a few occasions. Nothing definite can be said as to whether this associated organism is the real cause of the mosaic. Further, even assuming that it is the cause of mosaic, it is not as yet possible to explain the capricious behaviour of the cultures in reproducing the mosaic in a large percentage of the inoculated plants on some occasions while at other times only a small percentage of the inoculated plants show symptoms of mosaic.

It was considered, therefore, desirable to study this organism in greater detail before entering into a discussion of (*a*) the debatable question whether this organism is concerned in causing mosaic or (*b*) the reasons why its cultures fail to reproduce mosaic on inoculation into plants, assuming of course, without having proved it as yet, that this organism may be the real cause of mosaic. The organism has been

tentatively identified by us as *B. cereus* which showed pellucid dots on agar cultures, the cause and origin of which dots had not been so far satisfactorily explained, some considering the dots to be the result of a bacteriolytic principle or bacteriophage present in the agar cultures, others considering them to be 'the places where the culture is in virus form'.

We have been unable to demonstrate the existence of any bacteriolytic principle as all the methods which we used to test the existence of the lytic principle gave always negative results.

We next tried the technique recently developed by Hadley and others for the study of the dissociation forms of bacteria and succeeded in finding that the organism isolated by us from mosaic-affected plants, dissociated into several variants which can be stabilised, that is, isolated and kept in culture without changing to other forms or reversion to the original forms.

After comparing these dissociative forms with the variants obtained from other organisms we have been able to give their equivalent names to some of the forms isolated by us. They are as follows :—

S (smooth) form with pellucid dots in its agar culture, is a spore-former, and motile by peritrichic flagella. It dissociates spontaneously into all the forms named below and is, therefore, the original or mother form of all the other variants.

(1) Swollen big coccus form called *A* by us but not yet given any equivalent name.

(2) Coccal rod form named '*B*' by us, also not yet identified with similar form of any other organism.

(3) Small cocci equivalent to Hadley's *G* type of Shiga dysentery bacillus.

(4) *R* (rough) form is non-sporulating, non-motile and without pellucid dots in its agar culture. This form has not been previously isolated from *B. cereus* by any other investigators who were searching for it. Its cultures are otherwise similar to the *S* form described above. This form dissociates in broth cultures only into the forms *B* and *G* described above but has not dissociated into swollen big coccus form or the filterable forms as yet.

(5) Filterable forms which are found in a stabilised condition when the broth cultures of *S* form are filtered after eight days' incubation. If filtered before this time the filterable forms have a tendency to revert to the *G* form and later to the original mother form *S*.

None of these variants show pellucid dots in their pure cultures on agar except the *S* form, but we have been able to demonstrate that the associated growth of *R* with either of the variants—coccal rod form (*B*) or coccal form (*G*)—gives rise to the pellucid dots similar to those observed in the cultures of the *S* form, probably because of the difference in transparency and refraction of the different kinds of colonies produced by these variants. Associated growth of the other variants swollen big coccus or the filterable forms with *R* form does not give rise to pellucid dots. Associated growth of any of the variants other than *R* among themselves has also not shown pellucid dots. The cultures of *S* or smooth form always show the pellucid dots. These dots can now be accounted for by the rapid and spontaneous dissociation of this sporulating mother form *S* into other variants in agar cultures, because when among the dissociated cells of different kinds, the colonies of *R* cells grow in close proximity to the colonies of coccal rod forms (*B*) or coccal forms (*G*) the pellucid dots will easily arise as has been already demonstrated by actual experiment. The reason why the *R* form culture does not show the pellucid dots is that it gives rise to the dissociative forms *B* and *G* only in liquid broth cultures and not in agar culture. It may not be out of place to suggest that there is a possibility of some of these several variants singly or in combination with others reproducing mosaic symptoms more uniformly than has been possible hitherto with cultures of the smooth variant. At least we consider that the question whether they do so or fail to reproduce mosaic symptoms is worthy of further study.

REFERENCES

Almquist, E. (1911). *Centralbl. f. Bakt. I. Orig.* **60**, 167.

———— (1922). *J. Inf. Dis.* **31**, 483.

Asheshov, I. N., Asheshov I., Khan, S., Lahiri, M. N. & Chatterjee, S. K. (1933). *Ind. J. Med. Res.* **10**, 1101.

Bergey, D. H. (1930). 'Manual of Determinative Bacteriology'. London, Baltimore.

Bewley, W. F. (1923). *Nature* **112**, 903.

———— (1931). *Nature* **127**, 3203.

Chester, F. D. (1903). *Annual Report of the Delware College, Agric. Expt. Stn.*, pp. 73-76.

Desai, S. V. (1933). *Ind. J. Agric. Sci.* **3**, 626.

Enderlein, G. (1925). 'Bacterien Cyclogenie'. Berlin und Leipzig. W. de Gruytor & Co. 390.

Joshi, N. V. (1920). *Mem. Dept. Agric. Ind.* (*Bact. Ser.*) **1**, 247.

Hadley, P. (1927). *J. Infect. Dis.* **40**, 1.

Hadley, P.; Delves, E. and Klimek, J. (1931). *J. Infect. Dis.* **48**, 1.

Hauduroy, P. (1927). *Compt. Rend. Soc. Biol.* **97**, 1392.

Hort, E. C. (1917). *Proc. Roy. Soc. London* (*Ser. B.*) **89**, 468.

Jones, D. H. (1920). *J. Bact.* **5**, 325.

Löhnis, F. and Smith, N. R. (1916). *J. Agric. Res.* **6**, 675.

———— (1921). *Nat. Acad. Sci.* **16**.

Mellon, R. (1919). *J. Bact.* **4**, 505.

———— (1926). *J. Bact.* **12**, 279.

———— (1927). *J. Bact.* **13**, 99.

Nelson, R. (1932). *Agric. Expt. Stn. Mich. State Coll. Tech. Bul.* No. **118**.

Stearn, E. W. and Stearn, A. E. (1933). *J. Bact.* **26**, 57.

THE BIRD ENEMIES OF THE COTTON LEAF ROLLER (*SYLEPTA DEROGATA* FB.) AT KHANEWAL, *MULTAN* (*PUNJAB*).

BY

M. AFZAL HUSAIN, M.A. (Cantab.)
Entomologist to Government, Punjab

AND

HEM RAJ BHALLA, B.Sc. (Ag.)
Agricultural Assistant, Department of Agriculture, Punjab

(Received for publication on 8th May 1937)

Introductory

In 1930 a study of the bird enemies of the Desert Locust (*Schistocerca gregaria* Forsk.) was made in Ambala and the results contributed to this *Journal*, Vol. 1, Part V, October, 1931. In 1933-34 an opportunity occurred for a study of the bird enemies of another serious pest, *Sylepta derogata* Fb. The results of this study are presented in this paper.

In October 1933, a report was received of a serious outbreak of the Cotton Leaf Roller (*Sylepta derogata* Fb.) in the fields of American cotton, adjacent to the Khanewal (Punjab) Forest Plantation, and in the neighbouring villages. The pest had increased so enormously that entire plants were almost completely denuded of leaves. Acres upon acres of the American cotton were defoliated, and as far as eye could see, this cotton crop, otherwise quite well grown and bearing a large number of bolls, appeared ' blighted '. On the few leaves which were still present, as many as eight caterpillars per leaf were counted. In Chak* No. 15 Forest Area eight men, working for two hours, could easily pick up, from plants growing over an area of about fifteen square yards, four thousand of these caterpillars. In other words, the population of the Cotton Leaf Rollers per square yard of cotton crop was about 267. At this stage it was useless to adopt control measures because (i) the maximum damage had already been done, and (ii) further increase of the pest had stopped because of the setting in of hibernation among the caterpillars, on account of the low temperature.

As the surviving over-wintered caterpillars would be responsible for the intensity of attack during the following year, steps were taken to deal with these hibernating caterpillars. The part played by birds in checking this pest was of great significance.

---

*Chak means a village.

DATA

Birds occurring in the area were shot and their stomach contents examined.

The nomenclature adopted is that of Stuart Baker—the Fauna of British India.

The following abbreviations have been used in the statement below :—

Abundance of birds.
R = Rare.
C = Common.
CC = More common.
VC = Very common.
VVC = Abundant, *i.e.* in swarms.
B.C.G.A. = British Cotton Growing Association Farm.
S = *Sylepta derogata* Fb. caterpillar.

Status as beneficial birds.
m = Minor importance.
M = Major importance.
VM = Very important.
VVM = Extremely important.

The figures in square brackets indicate the serial number of the birds of each species shot and examined ; figures in circular brackets indicate the number of specimens discovered in the stomach contents.

Family name	Abundance status	Locality, date, stomach contents	Remarks
CORVIDÆ			
1. The Punjab Raven (*Corvus corax laurencei* Hume). (*Bara Kawa*).	CC/M	B. C. G. A. 1933. xi. 13. [1] *S.* (43) ; beetles (9) ; sprouting wheat grains (14). [2] *S.* (38) ; ants and insect remains ; sprouting wheat grains (28).	Took *S.* from ground and plant.
2. The Common Indian House Crow (*Corvus splendens splendens* Vieill.). (*Kawa*).	VC/VM	B. C. G. A. and 84/10R 1933. xi. 5. [1] Mainly sprouting wheat grains. 1933. xi. 7. [2] *S.* (45) ; beetles (2) ; insect remains ; sprouting wheat grains (12). 1933. xi. 9. [3-4] *S.* (15, 29) ; insect remains ; sprouting wheat grains (24, 44). 1933. xi. 10. [5-6] *S.* (27, 43) ; insect remains ; sprouting wheat grains (61, 79).	Took *S.* from ground and plant.
3. The Indian Tree-pie (*Dendrocitta rufa rufa* Latham). (*Mutri or Mahalat*).	C/M	B. C. G. A. and 84/10 R. 1933. xi. 6. [1] Mainly Hymenoptera (Wasps). 1933. xi. 7. [2] *S.* (29) ; *Chrotogonus* spp. (3) ; beetle and insect remains. 1933. xi. 13. [3] *S.* (23) ; beetles (5) ; wasps (9) ; insect remains.	Took *S.* both from ground and plant.

Family name	Abundance status	Locality, date, stomach contents	Remarks
TIMALIIDÆ			
4. The Bengal Jungle Babbler (_Turdoides terricolor terricolor_ Hodgs.). (_Sori_).	CC/M	B. C. G. A. . . . . . 1933. xi. 6. [1] _S._ (7) ; ant and insect remains.	Took _S._ both from ground and plant.
		1933. xi. 7. [2] _S._ (21) ; ants and insect remains ; chari grains.	
		1933. xi. 14. [3] _S._ (13) ; insect remains and wheat grains.	
		1934. ix. 29. [4] _S._ (2) ; ants and insect remains ; wheat grains (4).	
		[5] Beetles (2) ; insect remains ; wheat grain (1).	
		1934. xii. 11. [6] _S._ (2) and insect remains.	
5. The Common Babbler (_Argya caudata caudata_ Dumont). (_Kaira_ or _chhoti sori_).	CC/M	B. C. G. A. and 84/10R. . . 1933. xi. 18. [1] _S._ (7) ; ants (8) ; beetles (2) ; wheat grains (13).	Took _S._ both from ground and plant.
		[2] _S._ (11) ; insect remains ; sprouting wheat grains (5).	
		1934. x. 4. [3] Ants and insect remains ; weed seeds.	
		[4] _S._ (2) ; insect remains ; wheat grains.	
		1934. xii. 11. [5] _S._ (1) ; insect remains and weed seeds.	
PYCNONOTIDÆ			
6. The White-cheeked Bulbul (_Molpastes leucogenys leucogenys_ Gray). (_Bulbul_).	C/m	B. C. G. A. and 84/10 R . . 1933. xi. 5. [1] _S._ (4) ; _sunn_* seeds (11) and other weed seeds.	Took _S._ from plant.
		1933. xi. 14. [2] _S._ (6) ; chari grains (2).	
TURDIDÆ			
7. The Northern Indian Stone Chat (_Saxicola caprata bicolor_ Sykes). (_Kali pidi_).	C/m	B. C. G. A. . . . . . 1933. xi. 8. [1] _S._ (3) ; ants and insect remains.	Took _S._ from plant.
8. The Indian Bush Chat (_Saxicola torquata indica_ Blyth). (_Pidi_).	C/m	Chak 84/10R . . . . . 1933. xi. 8. [1] _S._ (3) ; ants (3) ; beetles (2) and insect remains.	Took _S._ from plant.
9. The Pied Chat (_Oenanthe picata_ Blyth). (_Pidi_).	C/m	Chak 84/10R . . . . . 1933. xi. 6. [1] Beetle elytron (5) ; ants and insect remains.	Took _S._ from plant.
		[2] _S._ (2) ; _Chrotogonus_ spp. (3) ; beetle and insect remains.	

*_Sunn_ is sann-hemp (_Crotalaria juncea_).

Family name	Abundance status	Locality, date, stomach contents	Remarks
TURDIDÆ—*contd.*			
10. The Eastern Indian Redstart (*Phœnicurus ochrurus rufiventris* Vieill.). (*Thir thira*).	C/m	Chak 85/10R . . . . 1933. xi. 15. [1] *S.* (5) ; beetle elytron (1) and insect remains.	Took *S.* from plant.
11. The Western Red-spotted Bluethroat (*Cyanosylvia suecica suecica* Linn.). (*Husseni pida*).	C/m	Chak 84/10R . . . . 1933. xi. 6. [1] *S.* (2) ; ants and insect remains.	Took *S.* from plant.
12. The Brown-backed Indian Robin (*Saxicoloides fulicata cambaiensis* Lath.). (*Dama*).	C/m	Chak 15 Forest area . . 1933. xi. 12. [1] *S.* (12) ; ants and insect remains.	Took *S.* from plant.
MUSCICAPIDÆ			
13. The White-browed Fantail Warble (*Rhipidura aureola aureola* Less.).	C/m	Chak 84/10R . . . . 1933. xi. 9. [1] *S.* (12) ; ants ; beetles and insect remains.	Took *S.* from plant.
LANIIDÆ			
14. The Indian Grey Shrike (*Lanius excubitor lahtora* Sykes). (*Lahtora*).	C/m	B. C. G. A. . . . . 1933. xi. 8. [1] *S.* (2) ; wasps (5) ; white ants and insect remains. 1933. xi. 10. [2] *S.* (6) ; beetle (1) ; *Chrotogonus* spp. (3) and insect remains.	Took *S.* from plant.
15. The Bay-backed Shrike (*Lanius vittatus* Valenc.). (*Chhota lahtora*).	C/m	B. C. G. A. . . . . 1933. xi. 6. [1] *S.* (11) ; *Chrotogonus* spp. (2) ; ants and insect remains.	Took *S.* from plant.
16. The Rufous-backed Shrike (*Lanius schach erythronotus* Vigors). (*Chhota lahtora*).	C/m	Chak 84/10 R . . . . 1933. xi. 14. [1] *S.* (5) ; wasp (1) ; beetles (2) ; ants and insect remains.	Took *S.* from plant.
DICRURIDÆ			
17. The Black Drongo (*Dicrurus macrocercus macrocercus* Vieill.). (*Chepu, Bhuchanga*).	CC/M	Chak 84/10R and 15 Forest . 1933. xi. 8. [1] Ants ; beetles and insect remains. 1933. xi. 10. [2] *S.* (10) ; beetles (3) ; *Chrotogonus* spp. (5) ; insect remains. 1933. xi. 15. [3] *S.* (15) ; *Chrotogonus* spp. (4) ; beetle and insect remains. 1934. iii. 9. [4] *S.*—moth remains ; *Chrotogonus* spp. (2) ; beetle and insect remains. 1934. iii. 12. [5] *S.*—moths remains ; beetles enormous. 1934. iii. 17. [6] *Chrotogonus* spp. mostly.	Took *S.* from plant.

Family name	Abundance status	Locality, date, stomach contents	Remarks
SYLVIIDÆ			
18. The Brown Willow Warbler (*Phylloscopus collybitus tristis* Blyth). (*Phootki*).	C/m	Chak 84/10R . . . . 1933. xi. 7. [1] *S.* (5) ; insect remains.	Took *S.* from plants.
		[2] *S.* (4) ; insect remains.	
19. The Green Willow Warbler (*Acanthopneuste nitidus nitidus* Blyth). (*Phootki*).	C/m	Chak 84/10R . . . . 1933. xi. 7. [1] *S.* (7) ; insect remains.	Took *S.* from plants.
		[2] *S.* (3).	
20. The Indian Streaked Wren Warbler (*Prinia gracilis lepida* Blyth). (*Khur-phootki*).	C/m	B. C. G. A. . . . . 1933. xi. 10. [1] *S.* (11) ; insect remains.	Took *S.* from plants.
STURNIDÆ			
21. The Rosy Pastor or Rose-coloured Starling. (*Pastor roseus* Linn.) (*Gulabi tiliar*).	VC/VM	Chak 2 Forest area . . . 1934. viii. 21. [1-2] *S.* (61, 89) ; ants and insect remains ; ficus fruits. [3-4] *S.* (53, 118) ; insect remains.	Took *S.* both from ground and plants.
22. The Himalayan Starling (*Sturnus vulgaris humii* Brooks). (*Tiliar*).	VVC/VVM	B. C. G. A. and Chak 15 Forest area. 1933. xi. 6. [1-3] *S.* (93-149) ; insect remains.	Took *S.* both from ground and plants.
		[4-17] *S.* (25-156) ; beetles (1-7) ; chari grains (1-15).	
		[18] *S.* (54) ; ants (3).	
		[19-20] *S.* (23, 70) ; chari grains (2, 5) ; wheat grains (2, 6).	
		1933. xi. 11. [21-22] *S.* (1, 53) ; insect remains.	
		[23-39] *S.* (16-143) ; beetles (1-7) ; insect remains.	
		[40-41] *S.* (119, 164) ; beetles (1, 2) ; *Chrotogonus* spp. (1).	
23. The Common Myna (*Acridotheres tristis tristis* Linn.). (*Lalri* or *sharak*).	VC/M	B. C. G. A. and 84/10R. 1933. xi. 6. [1] *S.* (3) ; insect remains ; wheat grains (29).	
		1933. xi. 7. [2] *S.* (29) ; ant (1) ; beetles (2) ; wheat grains (12) ; chari grains (15). [3] *S.* (9) ; insect remains ; wheat grains (9).	Took *S.* both from ground and plants.
		1933. xi. 9. [4] *S.* (18) ; insect remains ; wheat grains (13).	
		1933. xi. 10. [5] *S.* (25) ; beetles and ants ; wheat grains (5) ; chari grains (6).	
		1934. x. 4. [6] *S.* (3) ; beetle (1) ; *Chrotogonus* spp. (1) ; wheat grains (4).	
24. The Bank Myna (*Acridotheres ginginianus* Lath.). (*Ganga maina*).	C/m	B. C. G. A. . . . . 1933. xi. 10. [1] *S.* (26) ; *mung* (*Phaseolus mungo*) (4) ; wheat grains (6).	Took *S.* both from ground and plants.

Family name	Abundance status	Locality, date, stomach contents	Remarks
FRINGILLIDÆ			
25. The Indian House Sparrow (*Passer domesticus indicus* Jard. and Selby). (*Chiri* or *gouriya*).	VC/m	These birds were observed taking *S.* from *bhindi* plants growing in vegetable garden in E. A. C. Forests' bungalow, Piranwala.	Took *S.* from plant.
MOTACILLIDÆ			
26. The Indian White Wagtail (*Motacilla alba dukhunensis* Sykes). (*Dhobin*).	C/M	Chak 84/10R . . . . 1933. xi. 8. [1-2] *S.* (1, 2) ; ants and insect remains. 1933. xi. 10. [3-4] *S.* (4, 7) ; ants and insect remains. 1934. ix. 26. [5] *S.* (3) ; beetles and insect remains.	Took *S.* from ground.
27. The Indian Blue-headed Wagtail (*Motacilla flava beema* Sykes).	C/M	Chak 15 (Forest area). 1934. ix. 14. [1] *S.* (2) ; beetle (1) ; beetles and insect remains.	Took *S.* from ground.
28. The Indian Pipit (*Anthus richardi rufulus* Vieill.).	C/M	Chak 84/10R . . . . . 1933. xi. 7. [1] *S.* (15) ; beetle (1) ; insect remains. 1934. iii. 9. [2] *S.* (2) ; *Chrotogonus* spp. and insect remains.	Took *S.* from ground.
ALAUDIDÆ			
29. Franklin's Crested Lark (*Galerida cristata chendoola* Franklin). (*Chandool*).	CC/M	Chak 84/10R . . . 1933. xi. 7. [1-3] *S.* (5, 29) ; beetles ; ants and insect remains ; chari grains and other seeds. 1934. iii. 9. [4] *S.* (3) ; ants ; beetles and insect remains.	Took *S.* from ground.
CORACIIDÆ			
30. The Indian Roller (*Coracias benghalensis benghalensis* Linn.). (*Nilkanth*).	CC/M	B. C. G. A. . . . . 1933. xi. 6. [1] *S.* (8) ; other caterpillars (5) ; wasps (5) ; *Chrotogonus* spp. (2) ; insect remains. 1934. iii. 17. [2] *S.*—moths ; beetles and insect remains ; *Chrotogonus* spp. (5).	Took *S.* from plant.
MEROPIDÆ			
31. The Common Indian Bee-eater (*Merops orientalis orientalis* Lath.). (*Son chirri*).	CC/m	Chak 84/10R and 15 (Forest area). 1934. iii. 12. [1] Insect remains. [2] *S.*—moths and insect remains ; *Chrotogonus* spp. (2). 1934. iii. 17. [3] *S.*—moths and insect remains. 1934. iii. 24. [4] Beetles and insect remains.	Took *S.* at flight.

Family name	Abundance status	Locality, date stomach contents	Remarks
UPUPIDÆ			
32. The Indian Hoopoe (*Upupa epops orientalis* Stuart Baker). (*Hudhud*).	CC/M	B. C. G. A. . . . . . 1933. xi. 6. [1] *S.* (18) ; ants (2) ; beetle grubs (2) ; insect remains.	Took *S.* from ground.
PHASIANIDÆ			
33. The Common or Grey Quail (*Coturnix coturnix coturnix* Linn.). (*Bater*).	C/m	This bird was reported to be taking *S.* from fields at Chak 15 Forest Area, during the months of September and October.	Took *S.* from ground.
34. The Indian Black Partridge (*Francolinus francolinus asiæ* Bonap). (*Kala titar*).	C/M	Chak 15 Forest Area. . . 1933. xi. 12. [1-2] *S.* (18, 24) ; beetles (1, 2) ; grass and weed seeds and pebbles.	Took *S.* from ground.
35. The Northern Grey Partridge (*Francolinus pondicerianus interpositus* Hartert). (*Titar*).	C/M	Chak 15 Forest Area . . . 1933. xi. 12. [1-2] *S.* (9, 25) ; beetle and beetle grubs (1, 3) : grass seeds, weed seeds and pebbles.	Took *S.* from ground.
CHARADRIIDÆ			
36. The Indian Red-wattled Lapwing (*Lobivanellus indicus indicus* Bodd.). (*Tatiri*).	C/M	Chak 84/10R . . . . 1933. xi. 10. [1] *S.* (9) ; beetles, ants and insect remains.	Took *S.* from ground.

CONCLUSIONS

Besides poultry which entered the infested fields and picked up Leaf Rollers, thirty-six different species of birds belonging to seventeen families ate *Sylepta derogata* Fb. caterpillars.

Flocks of the Himalayan Starlings had come to the locality, and most of the other local birds, both insectivorous and omnivorous, were very busy throughout the day picking up Leaf Rollers. The following played an important part :

(1) The Himalayan Starling ; (2) the Rosy Pastor ; (3) the Mynas ; (4) the Common Indian House Crow ; (5) the Indian Pipit ; (6) Franklin's Crested Lark ; (7) the Babblers ; (8) the Black Drongo ; (9) the Shrikes ; (10) the Punjab Raven ; (11) the Common or Grey Quail and Partridges ; (12) the Indian Roller.

The Black Drongo, the Indian Roller and the Common Indian Bee-eater were seen sitting on dried cotton plants in March, 1934, and occasionally dashing for the Cotton Leaf Roller moths. The stomach contents of these birds showed the presence of these moths.

Of all the above-mentioned birds, the Himalayan Starling is *par excellence* a friend of man. It played havoc with the Cotton Leaf Rollers and must have been a factor of considerable importance in checking the pest. Thousands of these birds, in flocks, used to enter the attacked cotton fields and each bird picked up a large number of these insects, both active and hibernating, from the plants as well as from the ground. These birds were present in such large numbers that the place where they sat looked black. When on the wing the flocks of these birds appeared from a distance like a jet-black mass of smoke. There could not have been less than ten thousand of these birds in this particular locality.

From the analysis of the stomach contents it is clear that on an average the morning meal of each Starling consisted of eighty-seven Cotton Leaf Rollers (caterpillars) and the evening meal consisted of seventy-five caterpillars, a bird thus taking on an average 162 insects per day. The average population of active and hibernating caterpillars in an attacked cotton fields, in Chak No. 15 Forest Area, was 267 in October 1933, and it was reduced to 3·3 to 5·1. This decrease was to a great extent the result of the efforts of Starlings and other birds.

It was also reported that the Common or Grey Quail was seen in very large numbers in cotton fields in Chak No. 15 Forest area and it picked up a large number of these insects. The Indian Black Partridge and the Northern Grey Partridge used to come out of the forest and enter the adjoining cotton fields and pick up caterpillars of *Sylepta derogata* Fb.

Some of the birds such as the Punjab Raven, the Common Indian House Crow, the Bengal Jungle Babbler, the Common Babbler, the Himalayan Starling, the Common Myna and the Bank Myna took sprouting wheat from fields sown in November, 1933. Of these birds, the stomach contents of the Punjab Raven, the Common Indian House Crow and the Common Myna showed the presence of fairly large number of sprouting wheat grains (*i.e.*, 6 to 79 grains). A few of the birds such as the Bengal Jungle Babbler, the White-cheeked Bulbul, the Himalayan Starling, the Common Myna, Franklin's Crested Lark took a few *chari* (*Andropogon sorghum*) grains. The following three birds (the White-cheeked Bulbul, the Indian Black Partridge and the Northern-Indian Grey Partridge), in addition to *S. derogata*, took a few weed and grass seeds.

REFERENCES

Afzal Husain, M. and Bhalla, H. R. (1931). *Ind. J. Agric. Sci.* **1**, 609-619.

Baker, Stuart (1922—28). 'Fauna of British India Birds,' Vols. I to VII. Second edition.

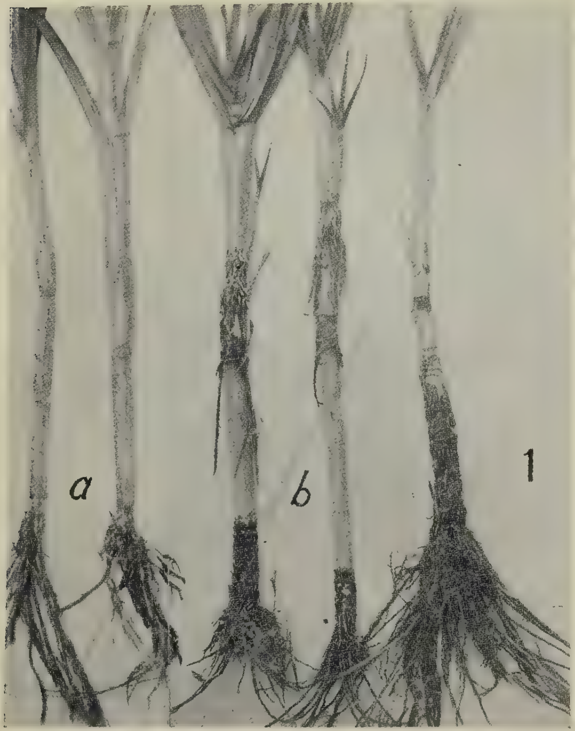


FIG. 1. (*a*) Two healthy plants. (*b*) Plants showing the earlier stages of the disease.

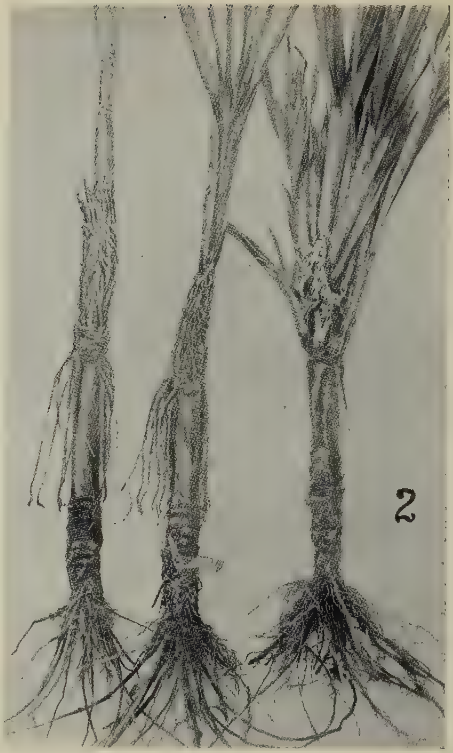


FIG. 2. Plants in advanced stage of the disease; lateral shoots of two plants removed to show adventitious roots developing near the apex.

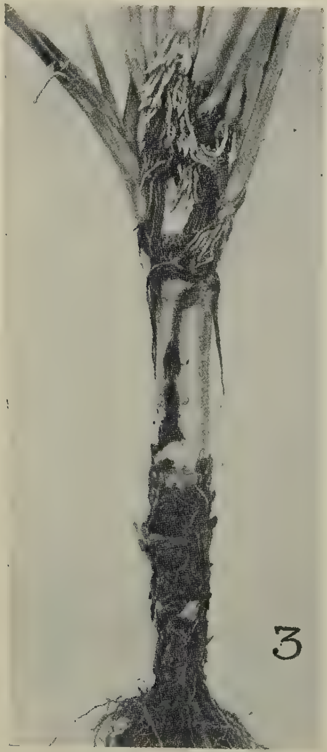


FIG. 3. Advanced stage of the disease. The basal part of the plant shows brownish patches; also showing the roots at the base of the lateral shoots.

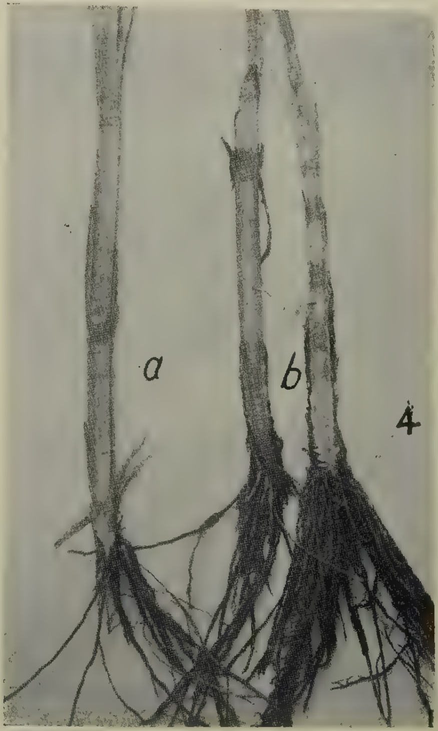


FIG. 4. (*a*) Longitudinally split healthy plant. (*b*) Longitudinally split diseased plants showing discoloured nodes.

A NEW DISEASE OF *ELEUSINE CORACANA GAERTN.*

BY

P. R. MEHTA

Assistant to the Imperial Mycologist, Imperial Agricultural Research Institute, New Delhi

AND

S. C. CHAKRAVARTY

(Received for publication on 2nd June 1937)

(With Plates LXVII and LXVIII)

A DISEASE of *Eleusine coracana* Gaertn., which seems to be hitherto unrecorded, was observed at Benares. Affected plants were stunted in growth, pale and tufted in appearance and characterised by the formation of numerous adventitious roots from the aerial nodes. They failed to develop the fertile shoots and, if they did, they were sterile. The internodes on the stem remained short. Tillering, however, was normal and the life period was not cut short ; in fact some diseased plants remained partially green till the harvest time of the healthy crop.

SYMPTOMS

The first indications of the disease were noticed in a plot with somewhat defective drainage after the crop was about one foot high when the growth slowed down very much. The plants which had looked slightly pale before were decidedly so now and presented a marked contrast to the healthy crop in an adjoining field. Leaf-blades of the younger plants showed numerous lesions from which *Piricularia* sp. was isolated. With the suppression of the apical growth there appeared numerous lateral shoots from the upper nodes (Plate LXVII, fig. 2). This does not occur normally in healthy plants. In the new lateral shoots, however, the same process of suppression of apical growth occurred, followed by formation on them of other lateral branches. In this way the plants got a tufted appearance.

On a closer examination it was found that the tufted appearance, in younger plants at least, was due also to the shortening of the internodes as a result of which the leaves stood close to one another and the leaf-sheaths seemed to enclose each other. The shortening of the internodes was in the entire or the middle part of the culm ; sometimes a zone of shortened internodes at the base was followed by an abnormally long internode and succeeded again by a zone of very short internodes near the middle of the culm (Plate LXVII, fig. 1). Many of the leaves,especially the lower ones, rapidly turned brown as also a few of the younger leaves at the apex. At a later stage of the disease the culms became brownish in patches and were slimy to the touch (Plate LXVII, fig. 3). The bigger lower leaves of the diseased plants tended to be upright and did not show the characteristic droop as occurs in healthy plants [Rangaswami Ayyangar, 1931].

The most characteristic feature of the disease was the formation of glistening white, usually unbranched, adventitious roots on the nodes, even from near the apex. They arose close together from the entire circumference of the nodes. There were as many as thirty-five roots on each node, each about one mm. in diameter near the base. They pierced through the leaf-sheaths in their downward course and remained closely adherent to one another through the entangled root hairs. The roots arising from different levels did not proceed straight downward but became firmly plaited ; this gave the mass of aerial roots a very characteristic appearance. These roots were unbranched, about three cm. in length and covered with root hairs along their entire length.

The roots which arose from the node adjacent to a long internode were very different from the above-described roots. These arose only from the narrow side of the laterally compressed culm. They were much longer (8 to 10 cm.), brownish, sparsely branched and closely adherent to one another and to the culm (Plate LXVII, figs. 1, 2). The adventitious roots from the basal nodes often reached the soil and there they formed branches. Lateral shoots also produced roots from their base which looked very much like adventitious roots on the nodes (Plate LXVII, figs. 2, 3).

A majority of diseased plants remained sterile and no fertile shoot was formed ; in a few cases a rudimentary fertile shoot with rudimentary ears developed but it never emerged out of the crown. It was noted that there was some correlation between the location of the root zone, the stunting of the plant and fertility. If the plants were very stunted they never formed fertile shoots ; these produced roots along their entire length, or along their greater part. On the other hand if the root zone was confined to two or three inches, near the base or the middle of the culm, a rudimentary fertile shoot frequently developed. But plants which had a very limited zone of adventitious roots near the base, and nowhere else, produced normal fertile shoot. In general the formation of adventitious roots anywhere except in a very limited zone near the ground level seemed to be detrimental to fertility. The lateral shoots remained leafy and infertile.

The underground root-system of a number of healthy and diseased plants was carefully dug out and exposed. No perceptible difference in their size or colour could be noted.

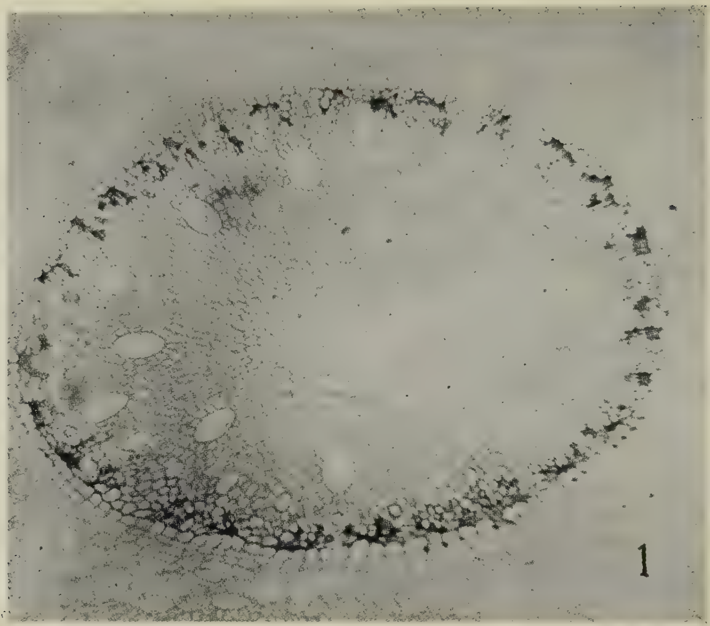


Fig. 1. Microphotograph of the stele of adventitious root. The dark patches mark the position of the phloem.

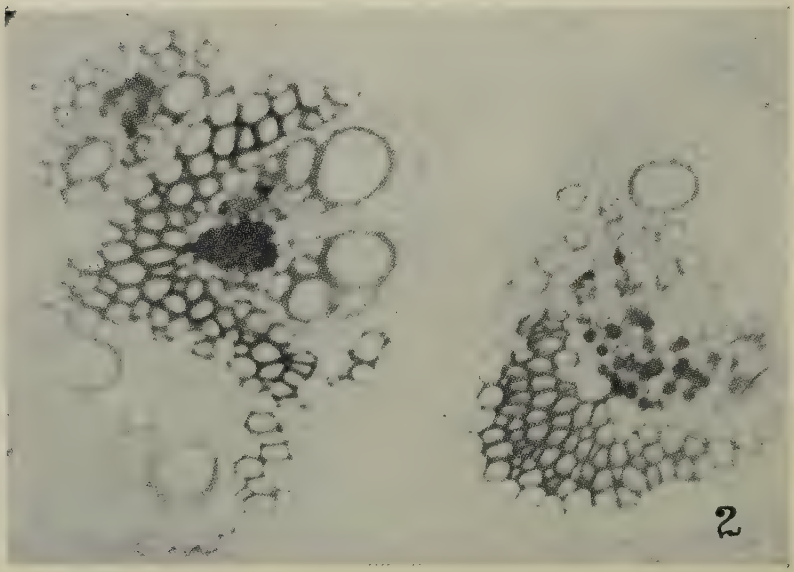


Fig. 2. Microphotograph of two vascular bundles of the stem. The phloem is affected in both.

On splitting open the culms it was observed that the interior of the diseased culms was discoloured. In the younger plants the internodes showed greyish streaks and the node was more or less completely discoloured (Plate LXVII, fig. 4). This grey colour gradually changed to yellowish brown in older plants.

MICROSCOPIC EXAMINATION

It was made by cutting microtome sections of the underground roots, nodes, internodes, and the adventitious roots. The material was fixed immediately after removing the plants from the fields. In order to make comparative study parts of healthy plants were also fixed.

The gross anatomy of the diseased plants was not much altered but the phloem in the different organs was discoloured and filled with bacteria, which were small rods, less than one micron in length and were difficult to stain with Ziehl's carbol-fuchsin or gentian violet. More satisfactory result was obtained by staining in eosin and borax-methylene blue as suggested by Mallory for Gram negative bacteria [McClung, 1929].

These bacteria were found in the phloem region of the underground roots of the diseased plants. The inter-cellular spaces took a bright stain indicating thereby that the bacteria were primarily inter-cellular. They were also noted in the cell cavities though rarely. Not all the phloem patches in the root were, however, affected ; sometimes the phloem on one side of the root (in transverse section) was healthy. The root hairs seemed to be normal, though in longitudinal sections the rootlets sometimes showed the bacteria while the main root was free.

Transverse sections through the plaited strands of adventitious roots showed that, owing to the lateral stress, their outline had become angular. These adventitious roots had the same type of anatomical organisation as the underground roots except that pith was parenchymatous and the xylem was poorly lignified. In these roots every patch of phloem was affected by the bacteria (Plate LXVIII, fig. 1).

Sections of the culm showed a similar localised distribution of the bacteria within the phloem (Plate LXVIII, fig. 2). Sections near the apex of the plants, which had developed adventitious roots near the apex, also showed bacteria in the phloem. Although there is a good deal of soft tissue in the apical region yet not one cell which was at some distance from the phloem showed the bacteria. In advanced cases of the disease the xylem cavities took up faint stain but bacteria were, however, never noted in their cavities.

Whether the bacteria are the cause of the disease or whether they are secondary invaders, is rather difficult to say at present. But their very localised distribution in the phloem of the different organs and their inter-cellular location, however, points to some specific relationship between them and the disease.

Other cases of disease in Gramineae are known where a prolific production of roots from aerial nodes is the common feature, as in 'Sereh' of sugarcane. In this case also there is arrested growth but both xylem and the phloem are, however, reddened. The Sereh disease is variously ascribed to virus, bacteria, and physiological causes. According to Bell [1929] sugarcane growing in acid soils which contain toxic amounts of soluble aluminium salts also produce roots from aerial nodes. The soil at Benares, however, is known to be slightly alkaline in reaction.

It may be of interest to state that a number of plants from the diseased plot when about six to eight inches high were removed to another plot and it showed less diseased plants, to the extent of only ten per cent ; whereas in the original plot almost all the plants were affected.

The exact cause of this new disease of *Eleusine coracana* is still under investigation.

SUMMARY

A new disease of *Eleusine coracana* was observed at Benares. Affected plants were stunted in growth, pale and tufted in appearance. The internodes were often very short and from the upper nodes there frequently developed numerous sterile lateral shoots. The most characteristic feature of the disease was the formation of numerous white adventitious roots from the aerial nodes. Diseased plants failed to develop the fertile shoot, and if they did, it was sterile. There was no sign of premature death nor was tillering affected. Microscopic examination revealed the presence of inter-cellular bacteria in the phloem of root and stem.

REFERENCES

Bell, A. F. (1929). *Queensland Bureau of Sugar Expt. Stn. Div. Pathology Bull.* **2**, 26.

McClung, C. E. (1929). 'Handbook of Microscopical Technique.' Paul B. Hoeber Inc.

Rangaswami Ayyangar, G. N. and Krishna Rao, P. (1931). *Ind. J. Agric. Sci*, **1**, 434.

A NEW CECIDOMYID PEST OF LINSEED IN INDIA

BY

HEM SINGH PRUTHI, M.Sc., Ph.D. (Cantab.), F.N.I.
Imperial Entomologist

AND

H. L. BHATIA, M.Sc.
Assistant to the Imperial Entomologist, Imperial Agricultural Research Institute, New Delhi

(Received for publication on 28th May 1937)

(With Plates LXIX and LXX)

CONTENTS

	Page.
I. Introductory	797
II. Systematic position	798
III. Nature of damage	799
IV. Life-history and description of various stages	799
V. Incidence of the pest on various varieties of linseed	804
VI. Summary	806
References	807

I. Introductory

The pest, which has been described as a new species (*Dasyneura lini*) by Barnes [1936], was first observed doing considerable damage to the flower buds of linseed at Pusa during the winter (January-March) of 1933. About fifty per cent of the buds were found infested. The affected buds did not open and consequently there was no seed-formation in them, thus entailing a heavy loss to the cultivator. From 1933 onwards the pest has been found on linseed at Pusa every year though its incidence has not been very high. After the shift of the Imperial Agricultural Research Institute to New Delhi, the linseed fields at Karnal were examined in the winter of 1937 and were also found to be infested with the pest. It is therefore very likely that *Dasyneura lini* is very widely distributed in the country. In view of the growing importance of linseed crop, the cultivation of which is being rapidly extended in all suitable parts of India, and the fact that numerous Cecidomyid species, *e.g.*, the Hessian fly, apple midge, clover midge [Barnes, 1927], the Gangai fly of paddy, etc., constitute very serious pests in India and other parts of the world, the new pest has been closely studied by us during the last two seasons at Pusa and the results of the investigation are reported in the following pages. There are still several lacunae in our knowledge of this pest and this preliminary account is being published so that entomologists working in various provinces of India may be able to identify and study the pest if it occurs in their areas.

It may be stated here that besides the Gangai pest of paddy (*Pachydiplosis oryzae*) mentioned above several Cecidomyids are known to occur on Indian crops, *e.g.*, sesamum (Gingelly), *cholam, juar, combu*, etc. Of these the Gingelly gall fly (*Asphondylia sesami* Felt) is important [Fletcher, 1920]. The genus *Dasyneura* itself to which the linseed fly belongs is represented by two species [Senior-White, 1928]. *D. gossypii* Felt [1916] is found in the flower buds of cotton which consequently get withered, fail to open and drop down [Fletcher, 1914, 1919]. It is however a minor pest. The other Indian species of *Dasyneura* is *D. mangiferae* Felt [1927] which was reared from mango flowers at Coimbatore in 1924. This species can hardly be considered to be a pest.

II. SYSTEMATIC POSITION OF THE PEST

According to the classification proposed by Kieffer [1913] or Felt [1925], our pest belongs to the genus *Dasyneura* Rond., which falls under the tribe Dasyneurinae of the sub-family Itonidinae, family Itonididae (Cecidomyidae).

According to Barnes [1936] the only other gall midge hitherto described from *Linum* is *D. sampaina* Tav., whose larvae produce oval terminal leaf-bud galls on *L. usitatissimum* and other species of *Linum* in Europe. Bequaert also recorded in 1914 a globular flower gall, each containing an orange-red larva on *L. strictum* in Algeria.

Barnes' specific diagnosis of *Dasyneura lini* is given below. A reference to Plate LXIX, figs. 9, 9*a* and 10, will facilitate the appreciation of the description :—

"*Male.*—Length slightly over 1 mm. Antennae two to thirteen-segmented ; first and second flagellar segments fused ; neck of first flagellar segments distinctly short, only slightly longer than broad, not half as long as basal portion of segment ; neck of second flagellar segment nearly three times as long as broad and nearly as long as basal portion of segment ; neck of third flagellar segment about three times as long as broad and as long as basal portion ; succeeding flagellar segments the same proportions, but gradually diminishing in size. Palpi : four segments, proportions indistinct, but distal segment distinctly longer than third. Thorax dark dorsally rest of thorax and abdomen fuscous. Wings : third vein reaching costa well before apex of wing ; fifth vein forked. Legs claws all toothed, empodium nearly as long as claws. Genitalia : basal clasp segment about one and a half times as long as broad, with a few long setae on inner surface, unusually broad ; distal clasp segment long, narrow, just over seven times as long as broad ; dorsal lamella with deep V-shaped emargination, each lobe rounded ; ventral lamella nearly as long as dorsal lamella, with shallow U-shaped emargination, each lobe narrow and rounded ; harpes long ; style reaching just beyond harpes ".

"*Female.*—Length about 1·5 mm. Antennae: scape and pedicel yellow, flagellar segments dark, 2/12, flagellar segments bead-like, progressively smaller. Palpi: four segments, proximal segment quadrate; 2nd about twice as long as 1st and slightly longer than broad; 3rd about three times as long as broad and nearly twice as long as 2nd; distal segment half again as long as 3rd and about six times as long as broad. Ovipositor pocket-shaped, largely protusible, otherwise about as in male".

III. NATURE OF DAMAGE

The midges are first seen in the field at Pusa early in January and the females start ovipositing soon afterwards. The eggs are laid between the folds of calyx of the young flower buds just where one sepal overlaps its neighbour (Plate LXIX, fig. 1). The buds at this time are green and the corolla is still lying folded within the calyx and is hardly visible from outside. The young larvae soon after hatching work their way inside the buds and start feeding on the essential organs of the developing flowers. Two to four larvae are usually obtained from a bud though in some cases up to ten larvae were found in a single bud. As a consequence of the feeding of the larvae the corolla of the infested buds presents an emaciated and crumpled appearance (Plate LXIX, fig. 2) and therefore the infested buds can be distinguished even from some distance. Such infested buds generally commence to appear in the field under normal weather conditions in the second week of January. The internal organs of the flowers also get shrivelled and withered (Plate LXIX, fig. 3) with the result that the buds do not open at all or open very slightly and therefore the seed does not set in them.

According to Wehrle [1928] and other workers similar damage is done by *Dasyneura leguminicola* Lint. to the flower buds of clover in several parts of America and Europe. Detmar [1927] describes similar damage to thistle by *D. gibsoni* Felt.

It may be added that at Pusa the flower buds of linseed are also infested by thrips. The thrips, however, do not seem to damage the buds in the same way as the Cecidomyid pest inasmuch as the buds in which the thrips alone were present did not present any crumpled and emaciated appearance. The buds infested with thrips alone had only the corolla somewhat decoloured.

IV. LIFE-HISTORY AND DESCRIPTION OF VARIOUS STAGES

The midges of *Dasyneura lini* are minute (1—1·5 mm.) and of beautiful orange colour (Plate LXIX, figs. 9, 10). For their structure the reader is referred to pp. 798-799 above. The females are specially active between 11 A.M. and 3 P.M., when the weather is bright and comparatively warm. It is at this time that the oviposition takes place. The males are common in the late afternoon and evenings. Both sexes are attracted to light in small numbers.

During February and first half of March the duration of various stages in the life-history of *Dasyneura lini* inside the laboratory at Pusa having average maximum and minimum temperatures of 77·5° F. and 72·0° F. respectively was found to be as follows :—

	Days
Egg	1-2
Larva	5-8
Pupa	4-6
Adult	4-6

Freshly laid eggs were generally met with in the field from the first week of January up to the third week of February and it appeared that three generations were completed from the beginning of January up to the middle of March. During January the incidence of the pest was always low, it was highest between 10th of February and 7th of March. The pest was found to disappear suddenly soon after the middle of March, by which time all varieties of linseed crop at Pusa are mature.

Efforts were made to ascertain as to what happens to the pest after the summer season sets in. For this purpose a large number of specimens were bred in the laboratory in the spring of 1936. About the middle of March while some larvae pupated and became adults in the normal way, a great majority of them went under soil debris, formed thin silken covers and entered a quiescent stage. The number of larvae entering such a resting stage increased as the weather became hotter and hotter. They remained in this condition up to the middle of August when we had to close down at Pusa and shift to New Delhi. It is very likely that in the field the species passes the hot dry season in this way. Several Cecidomyid species are known to have similarly prolonged larval life in the quiescent stage under adverse weather conditions. Wehrle [1928] found this period in the case of *Dasyneura leguminicola*, etc., to be about one year sometimes. The pea midge, *Contarinia pisi*, and several other midges in England pass whole of the winter in such a stage [Barnes, 1927]. It is highly probable that *Dasyneura lini* revives soon after the rains or in the beginning of autumn in the plains of India. The exact nature of its food plant or plants from this time up to the end of December when linseed buds become available has yet to be discovered. In this connection it is of interest to record that at Pusa we found the flowers of *Cajanus indicus* to be infested by a Cecidomyid fly very similar to *Dasyneura lini* during December and January (1935-36 and 1936-37). As the exact identification of this species is very important in reference to the alternate food plants of the pest, a good series of specimens of this form were sent to Dr. Barnes, the author of the species, *Dasyneura lini*. He replied (1st January 1937) as follows :—

> " Regarding the flies bred by you from *Cajanus indicus*, I cannot come to any very definite conclusions. They are exceedingly closely allied to *Dasyneura lini* but there are small differences, possibly covered by variation. I feel they are distinct species but

would very much like you to have tests made to see whether the species on *Linum* can mate (and produce offspring) with the midges from *Cajanus*. Also whether midges from *Cajanus* will breed on *Linum* and *vice versa*. One reason I think they are different species is that I know of only one species of midge that has been recorded from plants in different families ".

In accordance with Dr. Barnes' suggestions young larvae from *Cajanus* were put on linseed and *vice versa* during last January and February at Pusa and in both cases the larvae completed their development. Except that mating tests have not yet been made, it is evident that the two midges are identical and that *Cajanus indicus* is an alternate host of the linseed midge at Pusa.

Description of various stages.—The eggs are laid singly or in small loose batches of three to five eggs within the folds of the calyx where the sepals overlap one another (Plate LXIX, fig. 1). The female possesses a well-developed ovipositor. We found it very difficult to make the fly oviposit in captivity, therefore the total number of eggs laid by a single female and duration of oviposition could not be determined. The eggs when freshly laid are almost transparent but soon turn dirty white. The egg (Plate LXIX, fig. 4) is elongated (0·25 mm. × 0·07 mm.), often slightly but sometimes deeply curved and possesses a distinct micropyle. Its surface is smooth and without any marking or sculpture. It is somewhat sticky and therefore does not get dislodged ordinarily. In the laboratory the eggs were often found to hatch during the night or up to about 10 o'clock in the morning, and had an incubation period of at the most two days, as already stated.

The number of moults undergone by Cecidomyid larvae has not yet been fully established. Marchal [1897], Kieffer [1900], Anderson [1935], etc., divided the larval period into phases without any particular reference to the ecdyses undergone. In the case of *Cecidomyia destructor* Marchal described three phases :—(*i*) the freshly hatched larva ; (*ii*) the phase of nutrition and growth ; (*iii*) the quiescent and non-feeding stage just before pupation. Hamilton [1925] was the first author to definitely conclude that there are four instars in the case of *Monarthropalpus buxi* Lab. Metcalf [1933] working on *Dasyneura leguminicola* Lint. also found four instars. In the case of *Dasyneura lini* also there seem to be four instars, though we actually did not detect the moult separating the 2nd from the 3rd instar. These two instars are morphologically quite distinct as will be evident from the descriptions of various stages given below.

The morphology and anatomy of Cecidomyid larvae have been studied by numerous writers. The monographs on this subject by Marchal [1897] and Kieffer [1900] are well known. Williams [1910], Haseman [1930], Hamilton [1925], Metcalf [1933], Otter [1934] and Anderson [1935] described the morphology of individual species and have discussed the homologies of several larval organs, though some of these workers did not study the embryology of the larvae concerned. As homologies of organs cannot be satisfactorily discussed without a knowledge of the development of the organs and as the study of the embryology of *Dasyneura lini* is beyond the scope of this paper, we have refrained from discussing homologies in the description of the larva which follows :—

First instar larva.—The freshly hatched larva is about 0·3 mm. long and 0·1 mm. broad. The body segments are distinct. The head has a pair of antennae (' antennules ', ' palps ', ' tentacles ' of various authors). The head is conical in shape and is strengthened by a circular ring of thin chitin within. Between the head and the thorax there is, as in other Cecidomyid larvae, a supernumerary (neck) segment which is provided with a pair of small dark ' eye-spots '. These spots are crescentic in shape and are placed back to back. The head and neck segments can be retracted in the first thoracic segment. The three thoracic segments are followed by nine distinct abdominal segments, the ninth being much smaller than the previous one. The body of the larva is flattened dorso-ventrally, narrowed in the anterior region and is without any spines and papillae. It is almost transparent excepting for a yellow patch in the abdominal region. Metcalf [1933] and Otter [1933] noted a pair of open spiracles on the eighth abdominal segment in the species studied by them but we have failed to detect any open spiracles in the larva under report. The larva at this stage is very sluggish, slowly crawls from the sepal on which it hatched to the base of the essential organs of the flowers and rests there. It seems to feed very little at this time and the infested bud does not show any sign of damage. The first instar larva corresponds to the first larval phase of Marchal [1897]. When thirty to thirty-five hours old the larva moults and enters the second instar.

Second instar larva.—It is slightly more than one millimeter long and 0·3 mm. broad. It has nine pairs of open spiracles of which there is one pair on the prothorax and eight pairs on the first eight abdominal segments. The body is opaque, yellowish and without spines and papillae. The larva is moderately active in this stage.

Third instar larva (Plate LXIX, fig. 5).—The larva at this time measures about 1·25 mm. in length and 0·3 mm. in breadth. It is in this instar that the sternal spatula (' breast bone ', or ' anchor process ' of various authors) makes its appearance. This organ is characteristic of Cecidomyid larvae and has a great variety of shape in different genera and species [Kieffer, 1900]. It is a chitinous thickening of the ventral body wall along the median line. It is generally somewhat anchor shaped, the fork of the anchor pointing towards the head and the posterior portion of the shaft, being enclosed in a pocket-like invagination of the body wall. In *Dasyneura lini* the sternal spatula (*st. s.*) is almost Y-shaped (Plate LXX, fig. 2) and remains yellowish in the 3rd instar. Diverse functions have been ascribed to the sternal spatula [Sharp, 1918]. Giard [1893] considered it to be useful in the leaping movements of the full-grown larva. Marchal thought it to be helpful in pupation.

It is also in the 3rd instar that the rudiments of the segmental spines and papillae make their appearance. They remain rather inconspicuous in this stage and will be described in the account of the next instar hereafter.

The larva feeds most actively in the 3rd instar. In the beginning it is whitish but towards the end of this instar it acquires a pinkish tinge.

The second and third instars lasted about sixty to seventy-two hours under laboratory conditions at Pusa and correspond to the 2nd larval phase (of feeding and growth) of Marchal.

Fourth instar larva (Plate LXIX, fig. 6).—At this time the larva is about 2·0 mm. long and 0·5 mm. broad. It is of beautiful deep pink colour and moves about very actively but does not feed much. It is flattened dorso-ventrally. The antennae (*an*) are distinctly two segmented and the eye-spots (*e*) are very conspicuous (Plate LXX, fig. 1). The mouth is surrounded by four appendages, which are considered to be the future mouth parts by some authors. The sternal spatula is highly chitinized, brown in colour and distinctly Y-shaped (Plate LXX, fig. 2, *st. s*). The segmental spines and papillae are now very well developed and chitinized. There are four pairs of spines on the first thoracic and the eighth abdominal segments, two pairs being on the notum and one pair on each of the lateral sides (Plate LXX, figs. 1 and 3, *d. s.*,*l. s.*). Each of the 2nd and 3rd thoracic and the first seven abdominal segments has only two pairs of spines corresponding to the lateral spines of the prothoracic segment (Plate LXX, fig. 3). There are nine pairs of open spiracles, one on the prothorax and the rest on the first eight abdominal segments (Plate LXX, fig. 4, *sp.* 1-9). The thoracic and the last abdominal spiracles open on the dorsal side of the segment at some distance from the lateral lines, whereas the rest of the abdominal spiracles open in the dorso-lateral regions of the body close to the lateral sides.

In the fourth instar the larva has also series of conical pseudopods on the ventral side (Plate LXX, fig. 3, *p*). There are three such appendages, two well developed and one (median) small, on the 2nd and 3rd thoracic segments and four, two large and two (near the median line) inconspicuous, on each of the first seven abdominal segments.

The arrangement of spines and pseudopods varies in different species of Cecidomyid larvae as will be evident from the comparison of the description given above with those appended by Hamilton, Otter, etc., in the case of the larvae studied by them.

Of the internal organs of the larva of *D. lini*, the fat tissue is the most conspicuous. All the thoracic and abdominal segments have each a pair of fat lobes in the dorso-lateral regions. There is also a series of ventral fat lobes arranged segmentally along the median line (Plate LXX, fig. 5, *lf* and *vf*). This arrangement of fat tissue is almost similar to that found in *Cecidomyia destructor* and in *Dasyneura leguminicola* by Marchal [1897] and Metcalf [1933] respectively.

Pupation and the pupa.—When the 4th instar larva is about 2-3 days old, it makes its way out of the flower bud and crawls down the stem or drops on the soil under which it pupates inside a cottony pinkish cocoon (Plate LXIX, fig. 7). During January, February and first half of March the pupation takes place soon after the cocoon is formed. But after the middle of March the larvae as already described do not form a thick cocoon but prepare a thin silky cover under which they enter a long quiescent stage, revive and presumably pupate when favourable weather conditions return. The pupal cases are covered with minute particles of soil and therefore it is extremely difficult to collect them in the field. It is of interest to record that inside the laboratory the larvae sometimes pupated without forming a cocoon and the pupae thus found (Plate LXIX, fig. 8) were apparently quite normal and gave rise to normal flies.

The pupa (Plate LXIX, fig. 8) is about 1·25 mm. long and 0·5 mm. broad. It almost resembles in shape the numerous pupae figured by Marchal and Kieffer in their monographs already referred to. It is of deep pink colour, with the cephalic and thoracic regions tinged with orange. The legs are almost hyaline, and project beyond the tip of the abdomen. The head has a pair of small stiff spines and two fine long hairs above the eyes. The thorax bears a pair of medium-sized spines on its dorsal side.

The imago.—For the description of the adult flies (Plate LXIX, figs. 9, 9*a* and 10) the reader is referred to pp. 798-799, where both sexes have been described in detail.

V. INCIDENCE OF THE PEST ON VARIOUS VARIETIES OF LINSEED

The incidence of *Dasyneura lini* on various varieties of linseed grown in our experimental plots at Pusa during 1936 is given in Table I. Buds having crumpled corolla, a characteristic sign of infestation, were first observed about the middle of January in the field containing the early-flowering variety, H 68. Towards the end of this month the incidence in this variety was 1·9 per cent. At this time the other varieties under observation, *viz.*, the middle-flowering (T 12) and late-flowering (Australian 1-2 and Flax J W S), which had been sown at about the same time as H 68 did not show any attack and in fact the flower buds had not yet formed in them. The incidence in the early-flowering variety rose to 2·8 per cent by the 7th of February, soon after which seed-formation took place in this variety. At this time the middle-flowering variety (T 12) started flowering and in the second week of February showed 3·5 per cent incidence which went up to 5·0 per cent during the next week, soon after which the variety reached maturity. The late-flowering variety, Australian 1-2, started flowering in the second week of February and showed an incidence of 0·5 per cent; during the successive three weeks the incidence was 4, 19·5 and 22 per cent (7th of March). The Flax started flowering at about the same time as the Australian but had a much higher incidence throughout. It was nineteen per cent in the second week of February and went up to forty-seven per cent by the 7th of March after which seed-formation took place and the pest was no more observed on linseed.

TABLE I

Statement of the incidence of the linseed fly on various varieties of linseed at Pusa during 1936

Date of examination	Variety	No. of flower buds examined	No. of flower buds infested by larvae	Percentage of larval infestation	Remarks
15-30th January.	H 68 (early-flowering variety).	1,350	26	1·9	Crumpled corolla appeared in the field about 10th January, actual infestation evidently took place earlier.
1-7th February.	Ditto . .	1,200	34	2·8	Seed-formation took place in the second week.
8-15th February.	T 12 (middle-flowering variety).	200	7	3·5	Flowering started in the first week of February.
,, .	J W S Flax (late-flowering variety).	200	38	19·0	Ditto.
,, .	Australian 1-2 (late-flowering variety).	200	1	0·5	Just started flowering.
16-23rd February.	T 12 (middle-flowering variety).	300	15	5·0	
,, .	J W S Flax (late-flowering variety).	200	43	21·5	
,, .	Australian 1-2 (late-flowering variety).	200	8	4·0	
23-29th February.	J W S Flax (late-flowering variety).	400	136	34·5	
,, .	Australian 1-2 (late-flowering variety).	400	78	19·5	
1-7th March.	J W S Flax (late-flowering variety).	100	47	47·0	
,, .	Australian 1-2 (late-flowering variety).	100	22	22·0	

From the foregoing it will be evident that the incidence of the pest on various varieties was very much dependent on the time when they flowered. As stated in section IV on life-history, the pest starts breeding most actively about the middle of February. The early-flowering variety started maturing by this time and probably therefore had the maximum incidence of 2·8 per cent only. In the middle-flowering variety which was in bloom during the second and third weeks of February, the maximum incidence was five per cent. In the late-flowering varieties, the Australian 1-2 and Flax, in which the flowering went on up to the end of the first week of March, the maximum incidence was 22·0 per cent and 47·0 per cent respectively. Furthermore it is evident that apart from the time of flowering certain varieties were relatively more susceptible. For example, of the Australian (No. 1-2) and J W S Flax, evidently the latter, having a maximum incidence of forty-seven per cent proved much more susceptible.

The above conclusions must be considered as tentative, as the observations on the comparative susceptibility of various varieties have been taken during one season only. It is likely that the varieties may behave differently at other localities in India or even at Pusa. Observations extending over several years and covering different localities can only warrant conclusive generalization on this point.

VI. Summary

Dasyneura lini Barnes, a new and hitherto unrecorded Cecidomyid species, attacking in its larval stage the flower buds of linseed, is described.

The midge lays eggs singly or in batches of three to five eggs in concealed positions in the folds of calyx of young, green buds. On hatching, the larvae suck the juices of the thalamus and other essential organs of the flower. As a result of the damage the corolla becomes crumpled, essential organs emaciated and setting of seed does not take place.

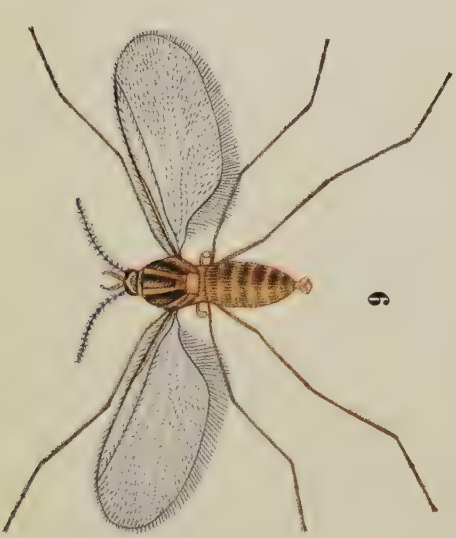
The larvae when full-grown crawl or drop down on the ground and spin cottony cocoons within which they pupate under soil.

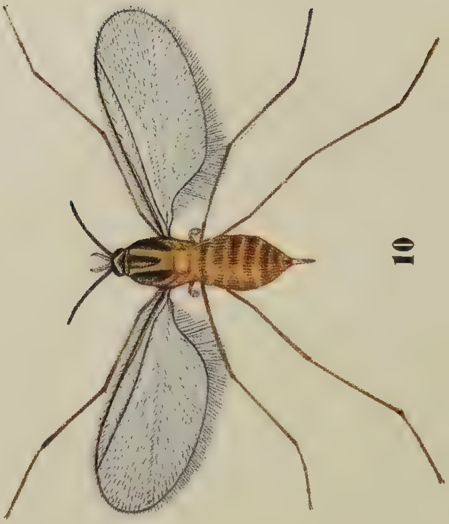
An account of the life-cycle and detailed description of various stages of the pest are appended.

It appears that *Cajanus indicus* is an alternate host of the pest at Pusa.

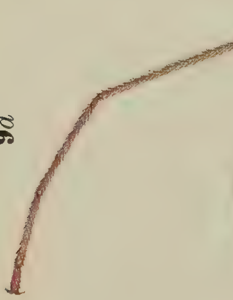
The pest seems to be widely distributed in India.

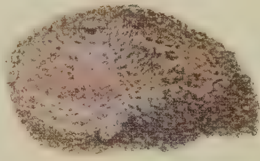
PLATE LXIX

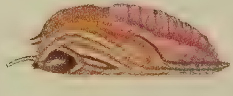

9

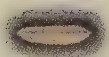

10

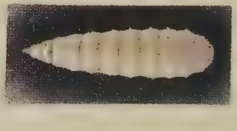
(*For explanation see p. 807*)

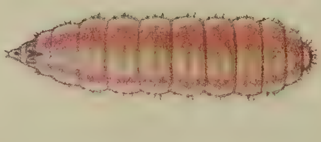

9a

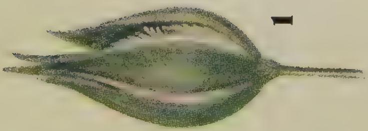

7

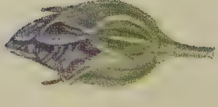

8

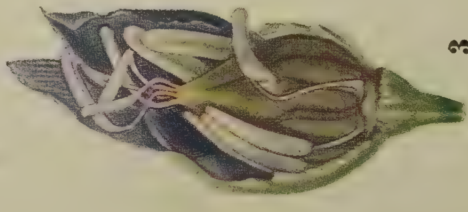

4


5


6


1


2


3

REFERENCES

Anderson, J. A. T. (1935). *Proc. Zool. Soc. Lond.* 405-420.

Barnes, H. F. (1927). *J. S. E. Agric. Coll. Wye*, **24**, 66-148.

——— (1936). *Ann. Mag. Nat. Hist.* **17** (10th Ser.), 273-274.

Detmar, F. (1927). *Bull. Ohio Agric. Expt. Stn.* **414**, 22-30.

Felt, E. P. (1916). *Canad. Ent.* **48**, 29.

——— (1925). *Bull. N. Y. St. Mus.* **257**, 1-239.

——— (1927). *Mem. Dept. Agric. Ind.* (*Ent. Ser.*), **10**, 1.

Fletcher, T. B. (1914), 'South Indian Insects', Madras.

——— (1919). *Bull. Agric. Res. Inst., Pusa*, No. **89**, 42.

——— (1920). *Rep. Proc. 3rd Ent. Meet. Pusa*, pp. 49-50.

Giard, A. (1893). *Bull. Soc. Ent. Fr.* **62**, 80-84.

Hamilton, C. C. (1925). *Bull. Md. Agric. Expt. Stn.* **272**.

Haseman, L. (1930). *J. Econ. Ent.* **23**, 316-322.

Kieffer, J. J. (1900). *Ann. Soc. Ent. Fr.* **69**, 181-472.

——— (1913). *Gen. Insec.* **152**, 346.

Marchal, P. (1897). *Ann. Soc. Ent. Fr.* **66**, 1-105.

Metcalf, M. E. (1933). *Proc. Zool. Soc. Lond.* 119-130.

Otter, G. W. (1934). *Parasit.* **26**, 582-593.

Senior-White, R. (1928). *Cat. Ind. Ins.* No. **15**, 4.

Sharp, D. (1918). 'Cambridge Nat. Hist., Insects, pt. II,' 4th edition, p. 460.

Wehrle, L. P. (1928). *Bull. Cornell Agric. Expt. Stn.* **481**, 1-35.

Williams, F. X. (1910). *Ann. Ent. Soc. Amer.* **3**, 45-60.

EXPLANATION OF PLATES

PLATE LXIX

FIG. 1. Flower bud with one of the sepals moved aside showing an egg clustar (× 3).

FIG. 2. Infested bud (× 2).

FIG. 3. Damaged bud partly dissected to show the essential organs (× 4).

FIG. 4. The egg highly magnified (× 40).

FIG. 5. The third instar larva (× 20).

FIG. 6. The fourth instar or full-grown larva (× 20).

FIG. 7. The cocoon (× 20).

FIG. 8. The Pupa (× 20).

FIG. 9. The male fly (× 20).

FIG. 9*a*. The hind leg of the fly (× 50).

FIG. 10. The female fly (× 20).

PLATE LXX

FIG. 1. The head and thorax of the full-grown larva, dorsal view (× 133).

FIG. 2. The head and thorax of the full-grown larva, ventral view (× 133).

FIG. 3. The full-grown larva showing pseudopods and spines, side view (× 37).

FIG. 4. The dorsal tracheal trunks and spiracles of the larva, partly diagrammatic (× 37).

FIG. 5. Preserved and cleared larva, showing the fat body within, dorsal view (× 28).

Key to lettering

a = anus, A_1—A_9 = nine abdominal segments, *p* = pseudopod, *an* = antenna, *d. s.* = dorsal spines, *e* = larval eye-spot, *H* = head, *H′* = supernumerary (neck) segment, *l. s.* = lateral spines, sp_1 = thoracic spiracles, sp_2 = first abdominal spiracles, sp_9 = eighth abdominal spiracles, *st. s.* = sternal spatula, T_1 = first thoracic segment, T_2 = second thoracic segment, T_3 = third thoracic segment, *t. r.* = tracheal trunk.

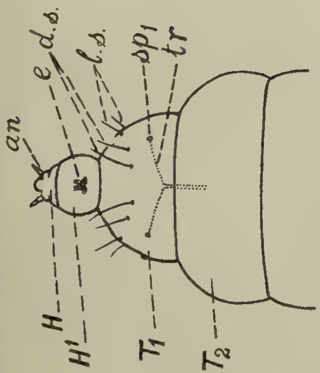

an
e
d.s.
l.s.
sp1
tr
H
H'
T1
T2

FIG. 1.

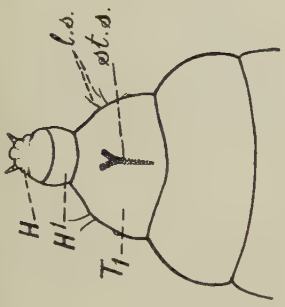


FIG. 2.

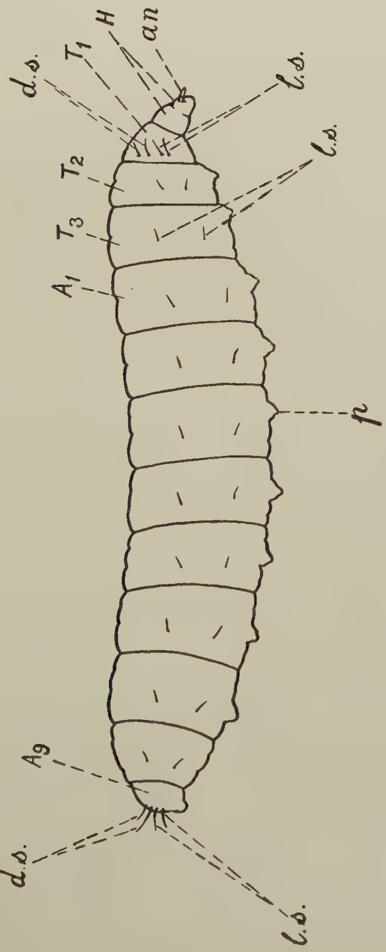

d.s.
T1
H
an
l.s.
T2
l.s.
T3
A1
p
A9
d.s.
l.s.

FIG. 3.

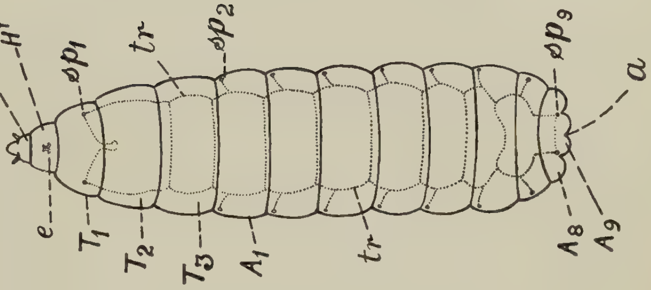

H
H'
sp1
tr
sp2
sp9
a
e
T1
T2
T3
A1
tr
A8
A9

FIG. 4.

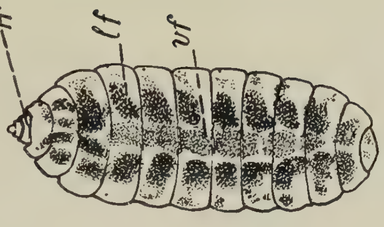


FIG. 5.

(*For explanation see page 808*)

REVIEWS

Drilling Mud: its Manufacture and Testing. By P. EVANS and A. REID. (*Transactions of the Mining and Geological Institute of India, Vol.* **32**, *December 1936*). Pp. 263+XXX. Price Rs. 13 or 19*s.* 6*d.* (inclusive of postage).

MODERN petroleum technology employs high speed rotary drilling by which it is possible to reach depths as great as ten to twelve thousand feet. It has been observed in practice, that clay-laden muddy water, possessing certain characteristics, has many decided advantages over clean water, as a circulating fluid in the tube of the rotary drill. This observation developed into an important feature of drilling practice in petroleum oil industry and special arrangements are made for the provision and supply of drilling mud of the requisite quality. The importance of drilling mud to the industry can be realised from the fact that over £25,000 were spent on the provision of drilling mud for a single rotary well.

As would be expected, the preparation and properties of fresh drilling mud and the regeneration of 'spent mud' have been the attention of investigators. Messrs. P. Evans and A. Reid have brought together in one volume the results of experiments carried out by the technical staff in the laboratories and fields of the Burma Oil Company. The publication deals with the preparation, physical and chemical properties and the testing of mud, an additional and important feature being the citation and discussion of relevant literature together with a description of the different apparatus employed.

The publication will be of interest to the soil worker in agriculture, although the problems under investigation and study are directly opposite to those of agriculture. The oil technologist is concerned with the manufacture of a stable mud suspension with a specified plastic flow, without setting into a jelly with age or use, while the agricultural soil worker is interested in the coagulation of what he calls refractory mud. The ends of both these classes of workers are certainly widely different but there are several in common in the means employed for the end. For example the studies on viscosity, plastic flow and thixotropy and the methods for the chemical and physical properties of clays are of interest to the soil scientist. [B. V. N.]

Annual Review of Biochemical and Allied Research in India, Vol. VII, 1936. By the Society of Biological Chemists, India, Hebbal P. O., Bangalore. Pp. 166. Price Rs. 2 or 3*s*. (postage extra).

THIS review contains seventeen articles most of which are more or less biochemical but the list also contains an article on soil physics and one on agricultural meteorology. Each article summarises the main lines of work and results in the particular field and is useful in bringing together in one volume work done by all research individuals and agencies, official and non-official. Merely as a bibliography, the volume is worth possessing and the short notes on the progress of the work cited are very useful. [W. B.]

The Plant Diseases of Great Britain. By G. C. AINSWORTH. (London: Chapman and Hall, Ltd., 1937). Pp. xii+273. Price 15*s*. net.

THIS is an interesting book. Its main interest lies in the endeavour of the author to make available in the compass of a single volume the key references for the principal plant diseases of Great Britain. In most cases the references have been carefully selected and annotated, and after a glance through the pages of the book one gets some idea of the amount of labour put in. To cite an example: the references to the literature dealing with potato blight caused by *Phytophthora infestans* (Mont.) de Bary have been so carefully selected that the reader gets a fairly good idea of the important published work. There is also an adequate subject and author index.

The usefulness of Dr. Ainsworth's book to plant pathologists in Great Britain is beyond doubt, and one can only hope that a similar work on plant diseases of India will be undertaken at no distant date. [B. N. U.]

Herbage Abstracts, Vol. VII, No. 1, March 1937. Published by the Imperial Bureau of Plant Genetics (Herbage Plants), Aberystwyth, Great Britain. Price 20*s*.

THE first issue of the seventh volume (March 1937) of this exceedingly useful publication has just appeared. It consists of 89 pages, excluding the indexes and is a comprehensive set of abstracts of every published paper connected distantly or remotely with herbage and forest plants throughout the world. The editors interpret their duties very liberally and we find amongst the titles such items as " Bird and Mammal communities and field borders in Northern Illixnois," " Plant-maps for the Netherlands 1 : 3,000,000. Part 1 ". Compiled by " The Institute for the Investigation of the Vegetation in the Netherlands " (I. V. O. N.) and " Reclamation of black-alkali soils with various kinds of sulphur ". The particular issue of *Herbage Abstracts* received is printed on one side of the paper only for the use of library and persons wishing to maintain card indexes. This means that the volume can be cut up for cards or insertion in catalogues. This publication is issued by the Imperial Bureau of Plant Genetics : Herbage Plants, Aberystwyth, Great Britain, which also publishes *Herbage Reviews.* The following are the rates of subscription :—

Herbage publication series.

Reviews—Abstracts—Bulletins.

	Shillings
Herbage Reviews alone	10
Herbage Abstracts (normal) either alone or with Herbage Reviews .	15
Herbage Abstracts (one side)* either alone or with Herbage Reviews .	20
Herbage Abstracts, both issues*	30
Herbage Abstracts, both issues* and Herbage Reviews . . .	35

* This is the issue just mentioned.

WOODHOUSE MEMORIAL PRIZE

THE following announcement has been received from the Director of Agriculture, Bihar :—

In memory of Mr. E. J. Woodhouse, late Economic Botanist and Principal of Sabour Agricultural College, who was killed in action in France in 1917, a biennial prize in the form of a silver medal and books of a combined value of Rs. 100 will be awarded to the writer of the best essay on a subject to be selected from the list noted below. The length of the essay should not exceed 4,000 words.

The competition is open to graduates of Indian Universities and to Diploma holders and Licentiates of recognized Agricultural Colleges in India who are not more than 30 years of age on the date of submission of their essays.

Papers should be forwarded to the Director of Agriculture, Bihar, Patna, before the 30th November, 1937.

Failing papers of sufficient merit no award will be made. Essays must be typewritten on one size of paper only.

LIST OF SUBJECTS

1. Hybrid vigour in plants and its significance in plant breeding and agriculture.
2. Agricultural problems of the Indian Sugar Industry and their solution.
3. The importance of purity and quality of seeds of farm crops and how to prevent their deterioration.

RECENT PUBLICATIONS OF THE IMPERIAL AGRICULTURAL BUREAUX

I. OBTAINABLE FROM THE IMPERIAL BUREAU OF SOIL SCIENCE, ROTHAMSTED EXPERIMENTAL STATION, HARPENDEN, HERTS

Periodical Abstracts

Lists of publications and papers on Soil Science published in the Empire Overseas in—

	Price *s.*	 *d.*
1933	1	0
1934	1	0
Soil Research in the British Empire published during 1935	1	0
Lists of Publications relating to Soils and Fertilisers—		
Published monthly, per annum, post free	10	0
Monthly Letters—		
Free to recipients, within the British Empire, of 'Publications relating to Soils and Fertilisers.' Subscription, outside the Empire, per annum	4	0
Recent Developments in Soil Analysis—		
Quarterly Supplement to the above publications. Separate copies each	0	6

Occasional Papers

Technical Communications—		
34. Tropical Soils in relation to Tropical Crops	2	6
Annual Report: For the year 1934-35	0	6
Bibliographies—		
Bibliography on Coffee	2	0
Catalogue of Journals and Periodicals in the Library of Rothamsted Experimental Station	2	0
Special Publication—		
The Katamorphism of Igneous Rocks under Humid Tropical Conditions (by the late Sir J. B. Harrison)	5	0
Bibliography of Soil Science, Fertilizers and General Agronomy, 1931-34	25	0

II. OBTAINABLE FROM THE IMPERIAL BUREAU OF ANIMAL HEALTH, VETERINARY RESEARCH LABORATORY, NEW HAW, WEYBRIDGE, SURREY

Abstracting Journal

The Veterinary Bulletin—		
1931. Vol. I. Quarterly (1st Number, April)	7	6
Annual Subscription	20	0
Subsequent Volumes. Monthly (1st Number, January)	5	0
Annual Subscription (postage paid)	40	0

Indexing Publication

	Price s.	Price d.
Index Veterinarius—Four issues a year. First issue April 1933— Annual Subscription (postage paid). Volumes I to III mimeographed, Volume IV onwards printed	100	0

III. Obtainable from the Imperial Bureau of Animal Nutrition, Rowett Research Institute, Bucksburn, Aberdeen

Journal

Nutrition Abstracts and Reviews. (Issued under the direction of the Imperial Agricultural Bureaux Council, the Medical Research Council and the Reid Library)—

	s.	d.
Subscription per volume of 4 numbers	42	0
Per single number	13	0

Occasional Papers

Technical Communications—

	s.	d.
7. Wheat : Pre-eminence as a Cereal Food, Nutritive Value ; Relation to Health and Disease	1	0

Occasional Communications—

1. The Effect of Climate on the Composition of Pasture Plants.

IV. Obtainable from the Imperial Bureau of Plant Genetics (for Crops other than Herbage), Plant Breeding Institute, School of Agriculture, Cambridge

Journal

Plant Breeding Abstracts—

	s.	d.
Quarterly. Annual Subscription	15	0
Single copies	5	0

Occasional Papers

	s.	d.
Indexes to Plant Breeding Abstracts—		
Subject Index to Vols. I to V of Plant Breeding Abstracts	5	0
Supplements to Plant Breeding Abstracts—		
Summary of Reports received from Countries exclusive of the British Empire, 1928-31. Supplement I	2	6
Summary of Reports received from Stations in the British Empire, 1932-1935. Supplement II	5	0
Technical Communications—		
Vernalization and Phasic Development of Plants (Joint Publication of the Imperial Bureaux of Plant Genetics)	10	0
Bibliographical Monographs—		
The Experimental Production of Haploids and Polyploids	5	0
Breeding Resistant Varieties, 1930-33 (Supplement)	2	0
An Outline of Cytological Technique for Plant Breeders.	1	6

V. Obtainable from the Imperial Bureau of Plant Genetics (Herbage Plants), Welsh Plant Breeding Station, Agricultural Buildings, Alexandra Road, Aberystwyth, Wales

Journals

	Price *s.*	*d.*
Herbage Abstracts—		
Quarterly. Annual Subscription	15	0
Single numbers	5	0
Herbage Reviews—		
Subscription is at present—Vol. 1 (1933), Vol. 2 (1934), Vol. 3 (1935), Vol. 4 (1936)—included in that to Herbage Abstracts		

Occasional Papers

Bulletins—		
17. Vernalization and Phasic Development of Plants. December, 1935. (Joint publication of the Imperial Bureaux of Plant Genetics)	10	0

VI. Obtainable from the Imperial Bureau of Fruit Production, East Malling Research Station, East Malling, Kent

Journal

Horticultural Abstracts—		
A quarterly abstract publication of current horticultural literature—		
Annual Subscription	15	0
Single copy	4	0

Technical Communications

8. Horticultural Aspects of Woolly Aphis Control together with a Survey of the Literature. R. M. Greenslade	2	6

Occasional Papers

3. Annotated Bibliography on Bitter-Pit. 1934	1	
Other Publications—		
Index to Volumes I-X of the *Journal of Pomology and Horticultural Science*, 1933. Compiled by Bureau, published by the Editors of the *Journal of Pomology and Horticultural Science*. Available from the Bureau	5	0
Old and New standpoints on senile degeneration, 1931. By A. P. C. Bijhouwer	0	6

VII. Obtainable from the Imperial Bureau of Animal Genetics, Institute of Animal Genetics, University of Edinburgh, King's Buildings, West Mains Road, Edinburgh

Journal

Animal Breeding Abstracts (Quarterly). Commencing April, 1933. Annual Subscription	15	0

Occasional Papers

	Price	
	s.	*d.*
Animal Breeding in the British Empire. A Survey of Research and Experiment, 1934	2	0

VIII. Obtainable from the Imperial Bureau of Agricultural Parasitology, Institute of Agricultural Parasitology, Winches Farm Drive, Hatfield Road, St. Albans, Herts

Journals

Bibliography of Helminthology. For the year 1933—		
Library Edition, bound in cloth	10	6
Stiff paper cover only	8	0
Helminthological Abstracts. (Issued in five or six parts per volume, each volume covering the literature of a single year.) Subscription per volume, payable in advance	30	0
Completed volumes bound in cloth	32	0

Occasional Papers

The Effects of some Natural Factors on the Second Ecdysis of Nematode Infective Larvae. By G. Lapage. 25 pp.	4	0
Notes and Memoranda—		
11. Recent Developments in the Control of *Heterodera marioni*. 6 pp.	1	6

Publications issued by the Imperial Institute of Entomology, 41, Queen's Gate, London, S. W. 7

Bulletin of Entomological Research.—Published quarterly and containing original articles on Economic Entomology. Issued post-free.

Annual subscription (payable in advance)	30	0

Review of Applied Entomology.—Consisting of abstracts or reviews of all current literature on Economic Entomology throughout the world. Published monthly in two series. Series 'A' dealing with Insect and other Arthropod pests of cultivated plants, forest trees and stored products of animal and vegetable origin, Series 'B' dealing with insects, ticks, etc., conveying disease or otherwise injurious to man and animals. Issued post-free.

	Series	
	'A'	'B'
	s.	*s.*
Annual subscription (payable in advance)	30	15
Vol. XXIII (1935)	42	21

	Price	
	s.	*d.*
Zoological Record—Part Insecta.—Published annually about July in each year and containing as complete a record as possible of the literature of the previous year chiefly from the systematic standpoint.		
Annual subscription (including postage)	15	6
Report of the Fourth Imperial Entomological Conference, 19-27th September 1935	4	0

Publications obtainable from the Imperial Mycological Institute, Kew, Surrey

Journal

Review of Applied Mycology.—Annual subscription, 12 monthly parts, with title page and index (post-free)	21	0
Single part	2	0
Title page and index	3	0
Report on the Third Imperial Mycological Conference, 1934	2	0

PUBLICATIONS OF THE IMPERIAL COUNCIL OF AGRICULTURAL RESEARCH, INDIA

[*Prices are inclusive of packing and Indian postage*]

1. Agriculture and Live-stock in India

ICAR. 1 A bi-monthly journal of agriculture and animal husbandry for the general reader interested in agriculture or live-stock in India or the tropics. (Established 1931.) Published in January, March, May, July, September and November. Prepayable subscription Rs. 6 or 9*s*. 9*d*. per annum. Price per part Rs. 2 or 3*s*. 6*d*. Volumes I to VI complete are available.

2. The Indian Journal of Agricultural Science

ICAR. 5 A bi-monthly scientific journal of agriculture and the allied sciences, mainly devoted to the publication of the results of original research and field experiments. (Established 1931.) Published in February, April, June, August, October and December. Prepayable subscription Rs. 15 or 24*s*. per annum. Price per part Rs. 3 or 5*s*. 3*d*. Volumes I to VI complete are available.

3. The Indian Journal of Veterinary Science and Animal Husbandry

ICAR. 6 A quarterly journal for the publication of scientific matter relating to the health, nutrition and breeding of live-stock. (Established 1931.) Published in March, June, September and December. Prepayable subscription Rs. 6 or 9*s*. 9*d*. Price per part Rs. 2 or 3*s*. 6*d*. Volumes I to VI complete are available.

4. Scientific Monographs of the Imperial Council of Agricultural Research

ICAR. 10-1 No. 1. The Fungi of India, by E. J. Butler, C.I.E., D.Sc., M.B., F.R.S and G. R. Bisby, Ph.D. (1931). Price Rs. 6-12-0 or 11*s*.

ICAR. 10-2 No. 2. Life-histories of Indian Microlepidoptera, Second Series : Alucitidae (Pterophoridae, Tortricina and Gelechiadae), by T. Bainbrigge Fletcher, R.N., F.L.S., F.E.S., F.Z.S. (1932). Price Rs. 3-4-0 or 5*s*. 6*d*.

ICAR. 10-3 No. 3. The Open-Pan System of White-Sugar Manufacture, by R. C. Srivastava, B.Sc. (1935, 2nd edition). Price Rs. 3-2-0 or 5*s*. 6*d*.

ICAR. 10-4 No. 4. Life-histories of Indian Microlepidoptera : Cosmopterygidae to Neopseustidae, by T. Bainbrigge Fletcher, R.N., F.L.S., F.E.S., F.Z.S. (1933). Price Rs. 4-8-0 or 7*s*. 6*d*.

ICAR. 10-5 No. 5. The Bombay Grasses, by E. J. Blatter, S.J., Ph.D., F.L.S. and C. McCann, F.L.S. Illustrated by R. K. Bhide. Price Rs. 20-12-0 or 32*s*. 6*d*.

ICAR. 10-6	No. 6. Helminth Parasites of the Domesticated Animals in India, by G. D. Bhalerao, M.Sc. Price Rs. 7-12-0 or 13*s*. 3*d*.
ICAR. 10-7	No. 7. Influence of Manures on the Wilt Disease of *Cajanus indicus* Spreng. and the Isolation of Types Resistant to the Disease, by W. McRae, M.A., D.Sc. (Edin.), F.L.S. and F. J. F. Shaw, D.Sc. (Lond.), A.R.C.S., F.L.S. (1933). Price Rs. 2-4-0 or 4*s*. 3*d*.
ICAR. 10-8	No. 8. The Silk Industry of Japan with Notes on Observations in the United States of America, England, France and Italy, by C. C. Ghosh, B.A., F.E.S. (1933). Price Rs. 4 or 6*s*. 9*d*.
ICAR. 10-9	No. 9. Mechanical Cultivation in India. A History of the Large-scale Experiments carried out by Burmah Shell Oil Storage and Distributing Company of India, Ltd., by C. P. G. Wade. Price Rs. 3-14-0 or 6*s*. 6*d*.
CAR. 10-10	No. 10. The Spotted Boll-worms of Cotton in South Gujarat, by B. P. Deshpande and N. T. Nadkarny. Price Rs. 5-14-0 or 9*s*. 6*d*.
ICAR. 10-11	No. 11. Investigations on the Course and Distribution of the Nerves supplying Levator anguli scapuli and Rhomboideus Muscles and the formation of the Phrenic Nerve in the Ox, with Observations on certain Anatomical Deviations, by H. N. Chelva Ayyangar, G.M.V.C. Price Rs. 4-10 or 7*s*. 9*d*.

5. Miscellaneous Bulletins of the Imperial Council of Agricultural Research

ICAR. 8-1	No. 1. List of Publications on Indian Entomology (1930), by the Imperial Entomologist, Pusa. Price As. 14 or 1*s*. 6*d*.
ICAR. 8-2	No. 2. List of Publications on Indian Entomology (1931), by the Imperial Entomologist, Pusa. Price As. 8 or 10*d*.
ICAR. 8-3	No. 3. List of Publications on Indian Entomology (1932), by the Imperial Entomologist, Pusa. Price As. 12 or 1*s*. 3*d*.
ICAR. 8-4	No. 4. Host Plant Index of Indo-Ceylonese Coccidae, by S. Ramachandran, L.Ag. and T. V. Ramakrishna Ayyar, B.A., Ph.D., F.Z.S. Price Re. 1-10-0 or 2*s*. 9*d*.
ICAR. 8-5	No. 5. List of Publications on Indian Entomology (1933), compiled by the Officiating Imperial Entomologist, Pusa. Price As. 9 or 1*s*.
ICAR. 8-6	No. 6. Bee-keeping, by C. C. Ghosh, B.A., F.E.S. (3rd Edition). Price Re. 1-14-0 or 3*s*. 3*d*.
ICAR. 8-7	No. 7. List of Publications on Indian Entomology (1934), compiled by the Officiating Imperial Entomologist and the Imperial Entomologist, Pusa. Price Re. 1-2-0 or 2*s*.
ICAR. 8-8	No. 8. Selected Clinical Articles, Part I, by G. K. Sharma, and R. L. Kaura. Price As. 8 or 10*d*.
ICAR. 8-9	No. 9. Statistical Methods and their Application to Agronomy. A Bibliography, by K. K. Guha Roy. Price Rs. 2-2-0 or 4*s*.
ICAR 8-10	No. 10. Diseases of Sugarcane and Methods for their Control, by L. S. Subramaniam. Price Re. 1-14-0 or 3*s*. 3*d*.
ICAR. 8-11	No. 11. Tables of Standard Errors of Mendelian Ratios, compiled by Swarn Singh Purewal, M.Sc., Ph.D. (Cornell), and P. Krishna Rao, L.Ag. Price As. 12 or 1*s*. 3*d*.
ICAR. 8-12	No. 12. List of Publications on the Botany of Indian Crops, Part II, for the Period 1928-32, by R. D. Bose. Price Rs. 3-6 or 5*s*. 9*d*.

ICAR. 8-13	No. 13. Two New Statistical Tables based upon Fisher's '*t*', by M. Vaidyanathan. Price As. 6 or 8*d*.
ICAR. 8-14	No. 14. List of Publications on Indian Entomology (1935), compiled by the Imperial Entomologist, Pusa. Price Re. 1-4 or 2*s*.
ICAR. 8-15	No. 15. Selected Clinical Articles, Part II, by G. K. Sharma, R. L. Kaura S. Ganapathy Iyer, G. S. Khan and S. Mangrulkar. (*In the Press*).
ICAR. 8-16	No. 16. Indian Grazing Conditions and the Mineral Contents of some Indian Fodders, by P. E. Lander, M.A., D.Sc., F.I.C., I.A.S (*In the Press.*)

6. Annual Report of the Imperial Council of Agricultural Research

ICAR. 12-31	Annual Report of the Imperial Council of Agricultural Research for the years 1929-30 and 1930-31. Price As. 12 or 1*s*. 3*d*.
ICAR. 12-32	Annual Report of the Imperial Council of Agricultural Research for the year 1931-32. Price As. 6 or 8*d*.
ICAR. 12-33	Annual Report of the Imperial Council of Agricultural Research for the year 1932-33. Price As. 6 or 8*d*. (Out of stock).
ICAR. 12-34	Annual Report of the Imperial Council of Agricultural Research for the year 1933-34. Price Re. 1-8 or 2*s*. 6*d*.
ICAR. 12-35	Annual Report of the Imperial Council of Agricultural Research for the year 1934-35. Price Re. 1 or 1*s*. 9*d*.
ICAR. 12-36	Annual Report of the Imperial Council of Agricultural Research for the year 1935-36. Price As. 14 or 1*s*. 6*d*.
ICAR. 12-37	Annual Report of the Imperial Council of Agricultural Research for the year 1936-37. Price Re. 1-2 or 2*s*.

7. Agriculture and Animal Husbandry in India (called Review of Agricultural Operations in India up to 1933)

ICAR. 9-29	Review of Agricultural Operations in India, 1928-29. Price Rs. 3-2-0 or 5*s*. 6*d*. (Out of stock).
ICAR. 9-31	Review of Agricultural Operations in India, 1929-31. Price Rs. 5 or 8*s*. 3*d*.
ICAR. 9-33	Review of Agricultural Operations in India, 1931-33. Price Rs. 5-12-0 or 9*s*. 6*d*.
	Agriculture and Animal Husbandry in India, 1933-34 and 1934-35.—
ICAR. 13-1-35	Part I.—Crop Production. Price Rs. 4-14 or 8*s*.
ICAR. 13-2-35	Part II.—Animal Husbandry. Price Re. 1 or 1*s*. 9*d*.

8. Proceedings of the Board of Agriculture and Animal Husbandry

ICAR. 2	Proceedings of the First Meeting of the Animal Husbandry Wing of the Board of Agriculture and Animal Husbandry held at New Delhi from the 20th to 23rd February 1933, with Appendices. Price Rs. 5-14-0 or 9*s*. 6*d*.
ICAR. 3	Proceedings of the First Meeting of the Crops and Soils Wing of the Board of Agriculture and Animal Husbandry in India held at Delhi from the 25th February to the 2nd March 1935, with Appendices Price Rs. 6 or 9*s*. 9*d*.

VRI. I	A Description of the Imperial Institute of Veterinary Research, Muktesar, and its Substation, the Imperial Veterinary Serum Institute, Izatnagar, by F. Ware, F.R.C.V.S. Price Re. 1-4-0 or 2*s*.
ARI. 7-187	The Production of Cigarette Tobacco by Flue-curing, by F. J. F. Shaw, C.I.E., D.Sc., A.R.C.S., F.L.S. and Kashi Ram. *Imp. Inst. Agri. Res. Pusa. Bull.* No. 187. Reprinted (1935). Price Re. 1 or 1*s*. 9*d*.
ICAR. 16	A Handbook of Statistics for Use in Plant Breeding and Agricultural Problems, by F. J. F. Shaw, C.I.E., D.Sc., A.R.C.S., F.L.S. Price Rs. 4-6-0 or 7*s*. 3*d*.

Copies of the above publications can be had from—

The Manager of Publications, Civil Lines, Delhi, and from any of the Agents, a list of whom will be found on the inside page of the front cover. Prices are inclusive of packing and Indian postage.

Purchasers not residing in Asia should apply to the High Commissioner for India, India House, Aldwych, London.

NOTE.—When indenting please give only the symbol preceding the name of the publication.

ERRATA

THE INDIAN JOURNAL OF AGRICULTURAL SCIENCE, VOL. 7, PART III, JUNE 1937

Page 375, letterpress of Fig. 2 should read "Illustrating the amount and rate of increase of leaf-curl in successive weeks after transplanting in normal-sown tobacco in 1935-36".

Page 379, line 7, *for* 'ew' (after 'containing') *read* 'few'.

GIPD—S 3—265 IC of AR—8-10-37—800.

ORIGINAL ARTICLES

OBSERVATIONS ON PHYLLODY OF SESAMUM IN BURMA

BY
D. RHIND
Economic Botanist, Burma

F. D. ODELL
Deputy Director of Agriculture, West Central Circle, Burma

AND

U THET SU
Mycologist, Burma

(Received for publication on 16th March 1937)

(With three text-figures)

I. Introduction

The sesamum crop in Burma is attacked every year to a variable extent by a disease which affects the reproductive organs. This disease has been described morphologically for sesamum in India by Roy [1931] and Pal and Nath [1935].

The disease in Burma was first described by McGibbon in 1924. Since 1923 the Agricultural Department in Burma has been studying this disease and a large number of experiments have been done, largely, it is true, with negative results. Examination of affected plants has failed to reveal any fungoid or bacterial infection and their presence is extremely unlikely since no necrotic lesions develop in plants affected with phyllody alone. Two theories as to the cause of the condition are held, one that it is due to a virus and the other that it is a purely physiological disturbance, sesamum being extremely affected by small changes in conditions. Pal and Nath [1935] have favoured the virus theory.

There has been some confusion of terminology of this disease and there does not appear to be much agreement as to its most suitable name. While the name phyllody is here used the same disease has been called sepalody, ‘ green flower ’, pothe (Burmese), phyllomania, etc. It is suggested that it would be well to retain only one name.

The disease appears to be distinct from the leaf-curl disease described from Tanganyika but a disease similar to leaf-curl has been observed in Burma bu not yet investigated.

The observations embodied in this paper are the result of investigations done by various officers of the Burma Department of Agriculture from 1923 onwards, some of which have been briefly mentioned in Annual Reports. They refer only to the early monsoon crop (long-day types). The winter (short-day) types are also affected to a smaller extent but there have so far been very few experiments on this crop.

II. Cultivators' opinions

In 1923 an enquiry was conducted in the East Central Circle (Pyinmana and Yamethin districts) with the co-operation of the Deputy Director into the supposed causes and predisposing conditions of phyllody in sesamum. From this enquiry several fairly definite opinions emerged. First, there was general agreement amongst cultivators that unseasonable sowing, more particularly early sowing, was conducive to the condition. In this area the most usual sowing time is about the middle of June and nearly all agreed that sowings in May or July gave badly affected crops. There was less agreement about the effect of rainfall though in general it was held that abnormally heavy rain as well as drought concurred with bad attacks.

There was almost complete agreement that early (monsoon) varieties [Rhind and Ba Thein, 1932] were more susceptible than late (winter) crops, while white-seeded types were held without exception to be worse than black or dark-seeded varieties.

There was not such general agreement on the influence of soil and fertility on the severity of the disease, but the general opinion was that on heavy soils and those likely to become hard after rain there is more of the disease while rich soils were considered to favour the disease more than poor soils.

III. Inheritance of susceptibility

In 1923 a large collection of sesamums was made from all over Burma and these were grown on the Tatkon Farm. Records were made of the percentage of phylloidal plants in each culture in 1924. In 1925 and 1926 cultures showing high percentages of phylloidal plants were discarded. An examination of the frequency curves of all varieties shown in Fig. 1 shows the rapid shift of the mode due to the elimination of those types which carried high susceptibility factors. The curve for 1925 appears to have three modes but this may be fortuitous. Great seasonal fluctuation in the severity of the disease is known to occur but it is considered that these curves show that selection for resistance to the disease has been rapidly effective. The cultures could not be considered homozygous in 1926 and this would explain the appearance of some lines with high percentages of diseased plants in that year.

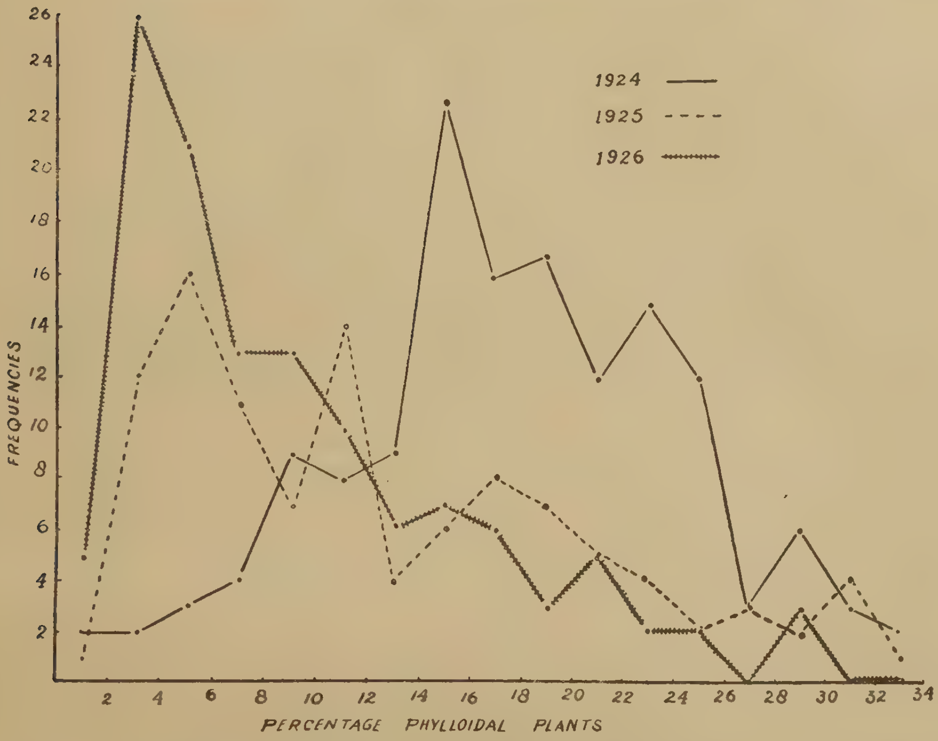


FIG. 1. Frequency distributions of phylloidal plants in three years crops and the effect of selection in displacing the modes towards the left.

Sesamum plants which develop phyllody late often produce seed which is viable though somewhat abnormal anatomically. In 1924 Odell [1925] sowed seeds from plants showing a slight degree of phyllody and found that the resulting crop averaged about one per cent of attacked plants as against a general mean of ten per cent for the rest of the sesamum on the farm. In 1925 [Odell, 1926] seed from the phylloidal plants of the 1924 trial was sown and again gave a crop almost free from the disease, freer than any of the nineteen local sesamums grown at the same time on the same farm. In 1927 this experiment was repeated and the results here produced do not show any reliable differences between the percentages of affected plants derived from phylloidal plants and those from healthy stock.

TABLE I*

Green flower inheritance test, Allanmyo Farm

Variety	Source of seed used							
	Affected plants 1927-28				Non-affected plants 1927-28			
	No. of plots	No. of plants	Plants affected by green flower	Per cent plants affected by green flower	No. of plots	No. of plants	Plants affected by green flower	Per cent plants affected by green flower
Shatgale Red	4	1940	152	7·8	4	2818	262	9·3
Shatgale White	4	1582	168	10·6	4	2944	373	12·7
Lemyaukgwa	4	1573	170	10·8	4	2165	215	9·9

* Reproduced from the Annual Report of the Allanmyo Agricultural Station, 1929.

There is nothing in these experiments to indicate that the disease is transmitted through the seed, though there are other results showing definitely that the degree of susceptibility is inherited.

IV. SEASONAL AND VARIETAL FLUCTUATIONS

Records have been kept of the maximum percentages of phyllody developed in the various cultures and multiplication crops grown on the Tatkon Farm since 1923. Owing to discarding for various agricultural reasons only four strains isolated in 1924 have been carried right through to the present time. Table II shows the percentages of phylloidal plants in these lines for the eleven years, 1925 to 1935.

TABLE II

Percentages of phylloidal plants in four lines of early sesamum for eleven years

Variety	1925	1926	1927	1928	1929	1930	1931	1932	1933	1934	1935	Mean
Gwagale 25-6 .	16·4	9·0	11·0	7·4	21·1	27·0	0·6	14·0	3·2	7·7	9·7	11·55
Taingdaung 25-181	17·4	0	12·0	14·5	19·4	25·5	0·8	7·4	2·8	13·8	23·3	12·44
Hnanni 25-160 .	1·8	0	4·0	1·9	34·4	16·0	0·8	5·3	1·9	3·4	12·9	7·49
Shithmyaung 25-187	2·7	2·0	10·0	0·4	32·1	24·0	0·6	16·8	6·2	3·6	18·7	10·64
Mean . .	9·67	2·75	9·25	6·05	26·75	23·12	0·70	10·89	3·52	7·12	16·15	10·534

From this table it can be seen that the annual fluctuation is very great. The seasonal effect is general for all four varieties grown under similar though not identical conditions, as shown by the curves in Fig. 2 which show agreement one with another.

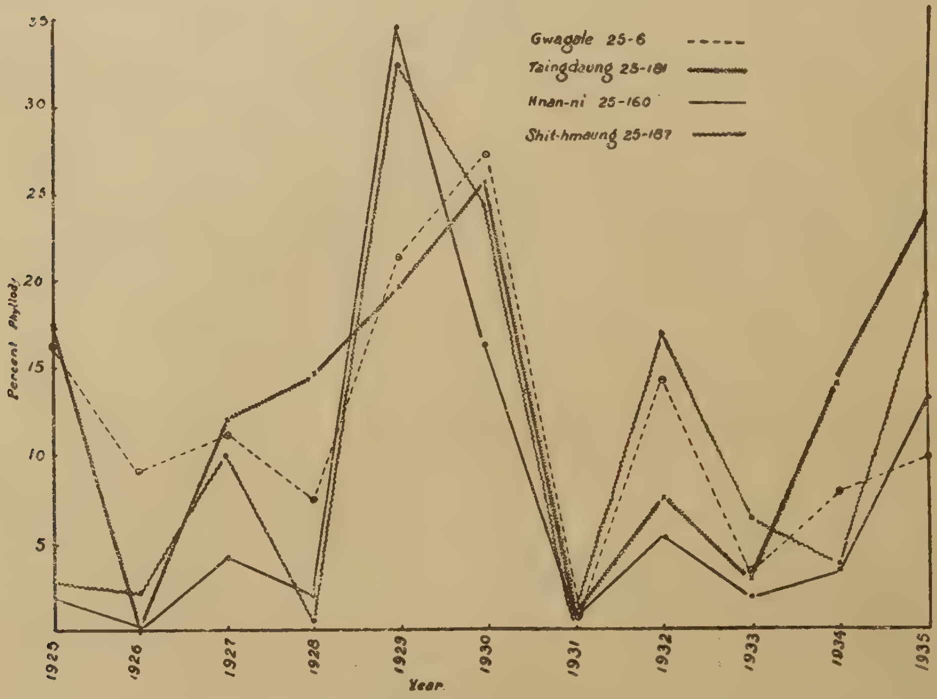


FIG. 2. Percentages of phylloidal plants in four cultures grown yearly at Tatkon from 1925 to 1935.

This is further emphasised when the correlations between the percentages of phylloidal plants in different strains are examined. These correlations are :—

Shithmyaung and Hnanni	+0·92	$P < 0{\cdot}01$
Shithmyaung and Taingdaung	+0·63	
Shithmyaung and Gwagale	+0·74	
Gwagale and Taingdaung	+0·72	
Gwagale and Hnanni	+0·73	
Taingdaung and Hnanni	+0·61	

The table also shows that distinct varietal differences exist, the variety Hnanni giving significantly lower percentages than the other three varieties. There is therefore one general factor affecting all plots in the same direction but unequally.

It may be noted that a bad season, which means the inclusion of a lot of seed from partly affected plants in the next season's sowing, does not entail a second bad crop. On the contrary, a season of high phyllody percentages is frequently followed by a season of low percentages and *vice versa.*

V. THE EFFECT OF SOWING DATE

The early sesamum crop in Burma is usually sown about the first half of June and opinion generally is that sowing too early or too late is conducive to a high degree of phyllody in the crop. To test this a number of experiments have been done by different workers on different farms in Burma. These experiments take the form of serial sowings at fixed intervals and counts of the affected plants resulting. The most elaborate of these were done by Odell and briefly reported in the Annual Reports of the Allanmyo Farm. Unfortunately all records of these experiments except one have been lost in a fire and in only one case is a detailed examination now possible.

Allanmyo experiment, 1930

In 1930 sowings were made daily in single rows from 15th May to 28th June inclusive, using the fully branched variety, Theikpan Hnannet Type [2 Rhind and Ba Thein (2), 1932]. Considering the daily sowings there is a definite decrease in phyllody percentage with later sowings, the regression coefficient being $-0{\cdot}29$ ($P<0{\cdot}01$). There is considerable irregularity, one sowing giving 2·0 per cent of affected plants while the sowing on the following day gave 15·7 per cent. When the results are grouped in four-day periods from the first sowing the curve is smoother (Fig. 3) and the regression coefficient rises to $-1{\cdot}255$ ($P<0{\cdot}01$). The correlation coefficient between date of sowing and percentage phyllody taking four-day totals is $-0{\cdot}888$ ($P<0{\cdot}01$). Attempts to relate the amount of phyllody with the rainfall failed to reveal any definite correlations. The coefficients were in most cases insignificant and differed in sign in consecutive sowings. Partial correlations eliminating sowing date effect were also mostly insignificant and of opposite signs.

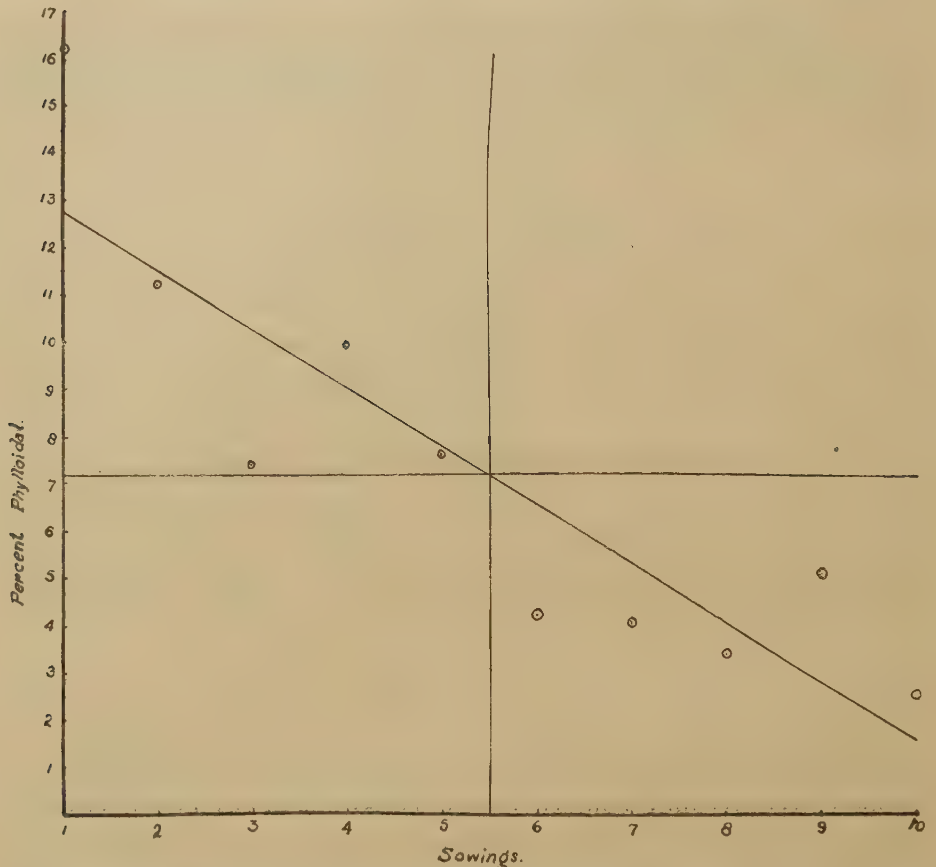


FIG. 3. Regression of percentage of phylloidal plants on sowing time. Sowings are means of four-day groups commencing 15th May 1930.

In 1930 this experiment was repeated at Magwe using the same variety and with sowings from 15th May to the end of September. Detailed records are no longer available and it is only possible to quote the brief report of the experimenter (F. D. O.) as published in the Annual Report of the Allanmyo Agricultural Station for 1931.

" The May sowings showed much vegetative growth with little fruiting and with a disposition to develop ' green flower '. Growth and fruiting was more normal and green flower progressively less in the sowings made towards the end of the month. Sowings made from the 1st to 15th June were normal fruiting and vegetative growth being proportionate and no green flower found.

In the 16th to 30th June sowings the plants again showed a departure from the normal, growth being progressively more stunted and fruiting less free in sowings made nearer the end of the month.

In the sowings made from July to the end of September the plants were very stunted and far from normal, flowering commencing in many cases when the plants were no more than six inches high.

The results confirm those previously obtained at Magwe and are in accord with those which have previously been recorded at Allanmyo."

It is to be noted that apart from the effect of sowing date on the percentage of phyllody a pronounced photoperiodic effect is to be expected as well [Rhind, 1935]. The variety used in the above test is a long-day type known to be very sensitive to small changes in day length. A difference of as little as one hour is known to have a very marked effect on reproduction.

Mycologist's experiments

In 1931 an experiment was laid down at Mandalay to ascertain the effect of sowing on different dates using the full-branched early variety Boktaung 25-225 (Type 34). Sowings were made fortnightly in randomised rows replicated six times. The results are reproduced in Table III.

TABLE III*

Percentage of 'green flowering' in sesamum sown on different dates

Date of sowing (1931)	13th May	1st June	23rd June	8th July	23rd July
Block I . . .	22·88	0	10·16†	4·81	1·03
II . . .	27·40	No germination	11·94†	5·97	0·93
III . . .	19·72	10·43	9·40	4·56	1·17
IV . . .	20·75	7·18	4·55	0·90	2·19
V . . .	14·48	No germination	7·08	2·26	2·24
VI . . .	19·38	Do.	10·76	8·44	3·25
Mean	20·77	5·87	8·95	4·49	1·80

* Reproduced from the Annual Report of the Mycologist for 1932.

† Estimated.

The results for the 1st June sowing were abnormally bad owing to poor germination and these are omitted from the analysis. For the two plots in Blocks I and II of the sowing on 23rd June interpolated values were used because germination failed and the analysis of variance with two missing plots proceeded with. The experiment passes the *z*-test and gives a standard error of 0·74. The differences of the means all exceed this more than three times and the experiment may be taken as showing a decrease in amount of phyllody with later sowings.

In 1932 an experiment at Mandalay with three sowings on 2nd, 16th and 30th May tended to show increasing amounts of phyllody with later sowing but the differences were not statistically reliable and the sowing dates not comparable with the 1931 experiment.

In 1933 the experiment was repeated with the results shown in Table IV.

TABLE IV

Percentage phyllody in sesamum sown on different dates at Mandalay

Sowing date (1933)	2nd May	11th May	16th May	20th May	9th June	13th June	Mean	S. E.
Per cent phyllody	17·23	18·05	17·88	11·57	7·02	4·17	12·65	3·61

The experiment was laid down as a 6×6 randomised block. The *z*-test is satisfied at the five per cent level. There is no appreciable difference between the first three sowings, nor is the fourth sowing definitely less affected by the disease, but the two June sowings are certainly less affected than the first three sowings in May. In the 1932 experiment there were only three sowings in May and these failed to attain significance, as is again the case with the May sowings in 1933. The June sowings in both 1931 and 1933 show reliable decreases in the number of plants affected.

These experiments taken as a whole show that in the case of long-day types normally sown as early monsoon crops there is a real tendency for early sowings to suffer more from phyllody than later sowings. At the same time very late sowings produce abnormalities of other kinds and lead to much reduced out-turns. For successful crops the sowing time appears to be restricted to the first three weeks of June. In Pal and Nath's [1935] experiment at Pusa the sowing time was 2nd August and this is approaching the normal sowing date of the late types. As was pointed out by Rhind and Ba Thein [1932] this late sowing masks the distinction between the long and short-day types since long-day types are merely rendered precocious by shortening the day. It was found that when the Pusa types were grown in the early monsoon (sown in first half of June) that they included several typical lates which were unable to flower under long days. The two strains (Types 3 and 7) used in this sowing date experiment by Pal and Nath have been proved here to be long-day types.

VI. INOCULATION EXPERIMENTS

To test the theory that the disease is produced by a virus inoculation and grafting experiments were undertaken. In 1926 and 1927 the Economic Botanist made a number of attempts to induce the disease artificially by the following four methods :—

1. Inoculation by implantation
2. Grafting (inarching)
3. Juice injection
4. Inter-connection with capillary tubes.

1. In the implantation method small slips of tissue from branches of diseased plants were introduced into incisions in the stems of unaffected plants and the wound bandaged. In 1926 four plants were treated in this way of which one developed the disease.

2. Branches of affected and unaffected plants growing in the same rows were grafted by inarching. In 1926 four grafts were made and one unaffected plant developed the disease, mostly in the lower part of the plant. In 1927 thirteen side-grafted plants failed to develop the disease in any of the healthy partners.

3. Juice was obtained from the affected parts of plants by crushing and was injected with a hypodermic syringe into apparently healthy plants. In 1927 sixty-four plants were so inoculated but none developed the disease.

4. Attempts were made in 1926 and 1927 to transmit the disease by connecting the stems of diseased and healthy plants with capillary tubes. The tubes were difficult to maintain in place in field plants on account of wind movements. Tubes were not sterilised and were empty at the time of insertion. In some cases they became filled with sap. In 1926 out of nine apparently healthy plants one developed phyllody, while in 1927 there were no successes with thirty-five treated plants.

All these experiments were done in the field without any precautions against chance infection and it is not possible to claim the three affected plants as successful inoculations.

In 1928 inoculation experiments were carried out at Allanmyo [Odell, 1929] where a plot of Bokni early sesamum free from phyllody was selected. The plot consisted of thirty-four rows. Alternate rows were inoculated with juice obtained by crushing the tops of phylloidal plants from adjacent plots. The result is shown in Table V.

TABLE V

Inoculation with juice from phylloidal plants, Allanmyo 1932

——	Inoculated	Not inoculated
Number of rows	17	17
Number of plants	200	199
Number of plants diseased	8	14

$\chi^2 = 1 \cdot 776$ P between $0 \cdot 1$ and $0 \cdot 2$

These results do not support the virus theory. It is to be noticed that the inoculations were done rather late, on 26th August by which time most of the plants in the test plot were flowering.

In 1929 the experiment was repeated [Odell, 1930] using three early types representing the unbranched, medium branched and full branched groups. They were sown in plots side by side (not randomised) on 23rd June. After forty days growth when the plants were about three feet high alternate rows of each variety were inoculated. The inoculations were done by making a deep scratch in the plants with a hypodermic needle filled with diluted juice obtained from crushing affected parts of older plants. The degree of dilution is not known nor the length of time which elapsed between extraction of the juice and inoculation. The defects of the method are realised, but as the total number of inoculations was 22,671 the figures are recorded in Table VI.

TABLE VI

Inoculation results for phyllody on early sesamums, 1929

—	Hlawagyi		Tetungale		Shwetachoung	
	Inoculated	Not inoculated	Inoculated	Not inoculated	Inoculated	Not inoculated
No. of rows .	60	60	73	73	84	85
No. of plants .	6240	6280	5311	5110	11120	9266
No. of plants affected	281	156	98	138	215	188
Per cent affected	4·50	2·48	1·84	2·70	1·93	2·03
χ^2 . . .	37·88		8·61		1·08	
P . . .	Very small		Less than 0·01		About 0·3	

Total inoculated . . . . =22671

,, not inoculated . . . . =20656

,, affected and inoculated . . . . = 594

,, ,, and not inoculated . . . . = 482

χ^2 . . . . =3·588

P . . . . $>0·05$

Although there appears to have been a significant increase in the percentage of affected plants in the variety Hlawagyi there also appears to have been the opposite effect in the variety Tetungale while Shwetachoung shows almost no effect. The combined results are inconclusive. The method of inoculation is recognised as likely to have been ineffective in the event of the virus being of a type not easily transmitted artificially. Furthermore, the possible effect of the mechanical injury caused by the inoculation was disregarded.

The inoculation experiments described by Pal and Nath [1935] also gave negative results but in the four cases where grafts took the results were positive. The authors, however, do not claim that these results are conclusive evidence in favour of the virus theory.

VII. Rainfall and phyllody

Enquiries amongst cultivators do not show much agreement as to the influence of weather conditions though it is generally held that too much or too little rain are conducive to a high percentage of phyllody in the sesamum crop. Examination of the rainfall records of the Tatkon Farm and comparison with the phyllody percentages in four lines over eleven years does not reveal any correlation, either when the total rainfall from sowing date to harvest (or to 31st August whichever was earlier) or when the fall within the first thirty days after sowing was considered. Neither could any relationship be discovered when the rainfall was taken in ten-day periods up to fifty days from sowing nor in twenty-day periods. There was a very slight indication that the disease was more prevalent in years of low total rainfall but the correlation coefficient of $-0 \cdot 15$ was insignificant.

It has been noticed that there appears to be a strong positive correlation between the incidence of the root-rot disease of early sesamum caused by *Macrophomina phaseoli* (Maubl.) Ashby and early sowing. This may result merely from the fact that early-sown plants are exposed to the risk of infection for a longer period but it may also mean that those plants which are disposed to the phylloidal condition are more susceptible to infection, and since these are more numerous in early sowings a higher percentage of root-rot cases results under these conditions.

VIII. Association of morphological characters with phyllody

The answers to the 1923 questionnaire all agreed that white-seeded types were more liable to phyllody than types having dark-coloured seeds. But seed colour itself is not independent of other characters and is in fact associated with branching habit which in turn is associated with life-period. Rhind and Ba Thein [1932] record the association between branching and sepalody (*i.e.*, phyllody) but since white seed is commonest with unbranched types the three characters—white seed, unbranched habit and short life-period—are all associated with high susceptibility to the disease. This is not to say that types having these characters and relatively immune to phyllody cannot be bred.

IX. SEED ABNORMALITIES

When plants are affected late in the growing period a number of capsules may be formed which outwardly show little or no abnormality. If the seeds contained in such apparently healthy fruits are dissected it is always found that the embryos are not normal. In embryos which are slightly affected the only abnormality may be a somewhat elongated and pointed radicle. In more seriously affected cases the cotyledons have become enlarged and crumpled, the edges convolute or undulate. The hypocotyl becomes elongated and distorted with pressure while the radicle develops with a long whip-like member twisted and tangled inside the seed-coat. The cotyledons in bad cases are usually thin and may be only a few cells thick. The general effect is that the embryos have not entered into a resting stage when formed but have at once continued growth and attempted germination but in an abnormal manner.

X. MINERAL METABOLISM OF DISEASED AND HEALTHY PLANTS

Klebs [1913] pointed out that high mineral nutrition tended to retard flowering and to stimulate vegetative growth. Estimates of the ash and certain mineral constituents of healthy and phylloidal sesamum plants from the same plot are given in Table VII.

TABLE VII

Analysis of ash of phylloidal and healthy sesamum plants from Tatkon

No.	Condition	Wt. of ash (grms.)	Wt. of sand (grms.)	Percentage of sand to ash	Wt. of Fe_2O_3 & Al_2O_3 (grms.)	Percentage of Fe_2O_3 & Al_2O_3 to ash	Wt. of Fe_2O_3 (grms.)	Percentage of Fe_2O_3 to ash	Percentage of Al_2O_3 to ash	Wt. of Al_2O_3 (grms.)
1	Phylloidal . .	1·95	0·3156	16·18	0·159	8·15	0·0039	0·2	7·95	0·1551
2	,, . .	2·66	0·335	12·59	0·522	19·62	0·0336	1·26	18·36	0·4884
3	,, . .	1·08	0·2068	19·13	0·100	9·26	0·0158	1·46	7·80	0·0842
4	,, . .	1·45	0·245	16·89	0·140	9·65	0·0138	0·95	8·70	0·1262
5	,, . .	0·915	0·2788	30·47	0·078	8·52	0·0178	1·94	6·58	0·0602
	Average for phylloidal	1·611	0·2762	19·05	0·2	11·04	0·0170	1·16	9·88	0·1828
1	Healthy . .	0·38	0·0918	24·16	0·0175	4·60	0·0059	1·55	3·05	0·0116
2	,, . .	0·32	0·0788	24·62	0·0135	4·22	0·0079	2·47	1·75	0·0056
3	,, . .	0·455	0·171	37·58	0·0295	6·48	0·0158	3·47	3·01	0·0137
4	,, . .	0·335	0·04	11·94	0·0095	2·83	0·0059	1·76	1·07	0·0036
5	,, . .	0·78	0·0882	11·30	0·0685	8·78	0·0184	2·36	6·42	0·0501
	Average for healthy	0·454	0·094	21·92	0·0277	5·38	0·0108	2·32	3·06	0·0169

These figures show that the diseased plants exceed the healthy in ash per cent significantly ($P < 0\cdot05$) while the amounts of ferric oxide and aluminium oxide are also higher in affected plants though the differences are not statistically significant. In a second series of phylloidal and healthy plants the following mean percentages were obtained :—

	Healthy	Phylloidal	
Per cent ash to dry weight	5·20	5·68	$P < 0\cdot01$
Per cent Fe_2O_3 to ash	1·46	2·07	P just exceeds 0·05
Per cent Al_2O_3 to ash	17·38	23·65	Do.

These figures are in conformity with those given in Table VII. A higher mineral metabolism is shown by the affected plants though since they were neighbours in the same plots it may be assumed that no large differences in mineral supply pertained.

XI. Discussion

The experiments described by previous workers to test the theory that the causative agent is a virus have not given conclusive results. The experiments here recorded are also indecisive, the most that can be said being that if there is a virus it is not of a type readily transmitted artificially.

The possibility that the disease is physiological is to some extent supported by the present observations though the virus theory is not wholly invalidated thereby.

There is no doubt that early sowing is conducive to the occurrence of the disease but this may merely mean that the plants are thereby brought into a period when the vector of the virus is more active or that they have been exposed to its attacks for a longer time. It must be noted, nevertheless, that early sowing imposes alterations in day length, and it is known that short-day types grown under long days do not flower but give luxuriant vegetative growth, with occasionally a few abortive flowers. The phylloidal plants can be arranged in a series from very slightly affected to those wholly sterile and foliose. In those slightly affected the early-formed capsules appear normal and the seeds also do not show symptoms, but the embryos are not normal. They show evidence that the period of dormancy has not supervened but instead growth has continued leading to the abnormal structures described in Section VIII. Worse affected plants show abnormal capsules and foliose structures on the placentae instead of ovules. Next in order of severity come plants in which the carpels are foliose, then the other whorls of the flower until finally the flower is replaced by a leafy shoot. The whole tendency is towards the vegetative condition, either from the start or by a regression from the reproductive stage. Lysenko has maintained that development proceeds in a series of distinct stages each of which must be completed before the subsequent stage can commence, but this view has been challenged by Ljubimenko [1935] and Ljubimenko and Sceglova [1936] who maintain the reversibility of the developmental processes. Botvinovskii and Sceglova [1935] have demonstrated the reversibility of the induction process in the case of *Perilla ocymoides* and Rhind [1935] recorded similar observations in the case of short-day sesamum. It has been suggested that the tissues laid down in the change-over periods between successive developmental phases would be abnormal and would lead to teratological structures. It seems that in the case of phyllody in sesamum we have an instance of the reversal to a greater or lesser extent of the progress of the reproductive phase or of the incomplete physiological differentiation of the reproductive tissues. The sesamum plant is known to be very susceptible to small changes in environment amongst which only day length has been studied to any extent. Although the difference between long and short-day types is known to be due principally to one factor, subsidiary factors are suspected and it is further to be remembered that none of the plants used in these observations could be regarded as truly homozygous, though they were sufficiently pure for ordinary agricultural purposes. It may therefore be imagined that varying conditions of light, temperature, moisture, soil, etc., acting on a mixture of gene complexes could serve to produce the disease in the sporadic manner in which it usually occurs.

XII. SUMMARY

1. The effect of selection in reducing the incidence of phyllody in mixed populations is demonstrated.

2. The results of sowing seed from affected plants gives no indication that the disease is transmitted through the seed.

3. The large seasonal fluctuations in the incidence of the disease are shown as well as the close correlations between the amounts of the disease in different strains in the same seasons.

4. Early sowing is shown to result in high percentages of affected plants.

5. The inoculation experiments described do not do more than show that if the causative agent is a virus then it is of a type not readily transmitted artificially.

6. While rainfall may be partly responsible there is no decisive evidence on this point. The tendency is for low rainfall to favour the disease.

7. The characters white seed-coat, unbranched habit and short life-period are associated with high susceptibility.

8. It is recorded that when slightly affected plants produce viable seed these seeds are abnormal and show failure of the normal onset of dormancy.

9. The higher mineral metabolism of diseased plants is recorded.

10. It is suggested that the condition may be accounted for by a failure of the normal progress of the reproductive phase induced by a combination of environmental conditions acting on complex undetermined genetic groupings and that it is a case of the return in varying degrees of the reproductive tissues to the vegetative condition. The possibility of a virus being the agent is not excluded wholly.

REFERENCES

Botvinovskii, V. V. and Sceglova, O. A. (1935). Review in *Bull. No. 17 of the Imperial Bureau of Plant Genetics* (*Herbage Plants*).

Klebs, G. (1913). *Handworterb. Naturw.* **4**, 276.

Ljubimenko, V. N. (1935). Review in *Bull. No. 17 of the Imperial Bureau of Plant Genetics* (*Herbage Plants*).

Ljubimenko, V. N. and Sceglova, O. A. (1936). Abstract in *Herbage Abstracts* **6**, 316.

Lysenko, T. D. (1935). Review in *Bull. No. 17 of the Imperial Bureau of Plant Genetics* (*Herbage Plants*).

McGibbon, M. (1924). *Rept. Econ. Bot., Burma*, 6.

Odell, F. D. (1925). *Rept. Allanmyo Agric. Stn.*, 4.

———— (1926). *Rept. Allanmyo Agric. Stn.*, 14.

———— (1929). *Rept. Allanmyo Agric. Stn.*, 9.

———— (1930). *Rept. Allanmyo Agric. Stn.*, 6.

———— (1931). *Rept. Allanmyo Agric. Stn.*, 7.

Pal, B. P. and Nath, P. (1935). *Ind. J. Agric. Sci.* **5**, 517.

Rhind, D. (1935). *Ind. J. Agric. Sci.* **5**, 729.

Rhind, D. and Ba Thein, U. (1932). *Ind. J. Agric. Sci.* **3**, 478.

Roy, S. C. (1931). *Agric. and Livestock in India* **1**, 282.

Thet Su, U (1932). *Rept. Mycol., Burma*, 4.

———— (1933). *Rept. Mycol., Burma*, 7.

———— (1934). *Rept. Operations Dept. Agric., Burma*, 27.

STUDIES IN INDIAN OIL-SEEDS

VI. SOME CORRELATIONS BETWEEN OIL-CONTENT AND OTHER CHARACTERS IN THE PUSA LINSEED HYBRIDS

BY

R. B. DESHPANDE, M.AG. (BOM.)
Officiating Special Research Assistant

AND

A. K. MALLIK, M.SC. (BOT.), B.SC. (AG.)
Post-Graduate Student, Botanical Section, Imperial Agricultural Research Institute, New Delhi

(Received for publication on 11th August 1937)

(With Plate LXXI)

CONTENTS

PAGE
I. INTRODUCTION 841
II. MATERIAL AND METHOD 843
III. CORRELATION BETWEEN OIL-CONTENT AND SEED-COLOUR . . . 844
IV. CORRELATION BETWEEN SEED-SIZE AND SEED-COLOUR . . . 845
V. CORRELATION BETWEEN OIL-CONTENT AND SEED-SIZE . . . 847
VI. DISCUSSION 847
VII. SUMMARY 848
REFERENCES 848

I. INTRODUCTION

LINSEED oil forms by far the major portion of the world's supply of drying oils used in the manufacture of paints, varnishes, oil-cloth, etc. The oil is extracted from the seed, the oil-content of which varies from about thirty-five to forty-five per cent.

In the production and export of this important agricultural commodity, India stands first within the British Empire; her only serious competitor is the Argentine. The Ottawa preference of ten per cent to Indian linseed has given a fillip to the Indian linseed trade, and the acreage under linseed is rapidly increasing. Research schemes to improve the quality and yield of Indian linseed are being launched. The recent announcement of an increased import duty (from £3-10 to £5 per ton) on linseed oil in the United Kingdom should also go a long way in stimulating this industry.

The principal object of economic work on linseed is improvement both in yield of seed per acre and in quality and quantity of oil. The oil-content being invisible is a difficult character for the plant breeder to handle. Its determination is a lengthy and laborious affair for which the breeder has to depend upon the analytical chemist. Consequently this character cannot be used as one of the bases of selection in the earlier stages of breeding work. It has often been the experience of breeders that a selection which is very commendable from all other points of view proves to be low in oil-content, when this is tested, and has to be abandoned. This involves a considerable loss of time, energy and money.

Realizing these difficulties, investigators have since long attempted to correlate this invisible character, oil-content, with some other visible character, such as seed-size, weight, volume, maturity, etc.

Working with Indian linseeds, Leather [1907] concluded from analyses of seeds obtained from several provinces in India that there was no appreciable relation between seed-size and oil-content. His conclusions may, however, be open to criticism as the samples with which he worked were grown under different environmental conditions.

Eyre and Fisher [1915] acknowledging " at least a parallelism " between seed-size and oil-content concluded that the variation in oil-content due to varietal differences masks any difference due to varying size of seed.

Howard and Khan [1924] stated, " Other things being equal, the larger seeds fetch a higher price. As would be expected, these differences in price depen 2 largely on oil-content ". They add, " To obtain a high oil-content, the cultivation of a large-seeded type would appear essential ".

Coleman and Fellows [1927] remark, " Test weight per bushel and oil-content have no close positive relationship." They say in another place, " In fact there is a tendency for samples of very high test weight to contain less oil than flax seed of lesser weight per bushel ". This, we think, suggests a positive correlation between seed-size and oil-content, for a linseed will give a higher test weight per bushel if it is smaller in size than one which is bolder, other things being equal.

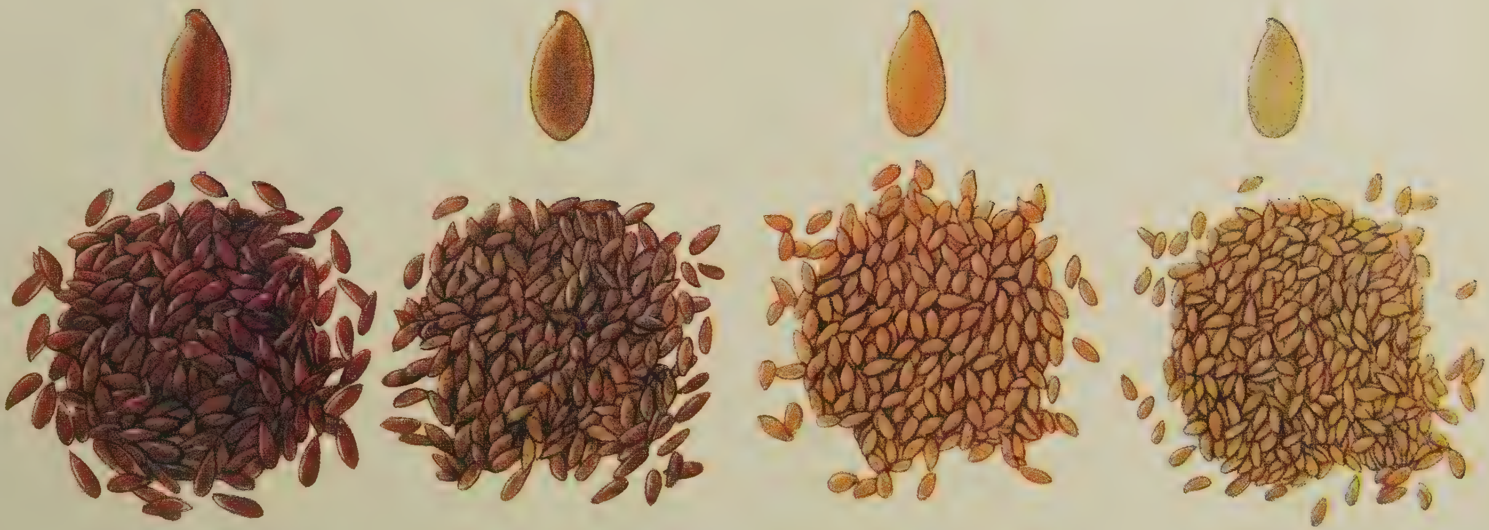
Johnson [1932] found a significant positive correlation between oil-content and weight of seeds. Dillman [1936] found high correlation between seed-size and oil-content of different varieties.

Regarding the relationship between oil-content and seed-colour, very little work seems to have been done. This may be because most of the work that has been carried out on this subject has been done in foreign countries, where the principal commercial varieties are brown-seeded.

Snyder [1905] found appreciable differences in the oil-content of flax seed of different shades of colour. The shades refer, we presume, to those within the brown colour.

PLATE LXXI

TYPES OF SEED COLOUR IN LINSEED



Brown Grey Fawn Yellow

Howard and Khan [1924] stated, "As regards the colour it is sometimes stated that the pale-seeded varieties are the most valuable. It is possible that this belief has partially arisen from the fact that all the yellow varieties have bold seeds and are, therefore, rich in oil-content ".

Coleman and Fellows [1927] did not find colour of seed to be an important index of quantity of oil in flax seed.

II. MATERIAL AND METHOD

Linseed has received special attention in the Botanical Section, Pusa, for some years. The classification of Indian linseeds by Howard and Khan [1924] was followed up by hybridization work by Shaw [1932]; this is being continued by the senior author of this paper. The object of this work is to combine the heavy-yielding capacity of the small-seeded linseeds of the Indo-Gangetic alluvium with the bold seed-size and high oil-content of the Central Indian types.

As a result of these investigations, a number of hybrids were evolved, some of which were selected for further breeding. A good habit (erect with good tillering), resistance to rust (*Melampsora lini*), and productivity were the main characters considered in making the preliminary selections. Oil-content could not be considered at this stage for the reasons already mentioned.

About ninety fixed hybrids were thus first selected, and their oil-content determined from year to year. As the work progressed, some further selections were made bringing the total up to 105. These hybrids vary in oil-content, seed-size, and seed-colour. The last character needs some explanation here. While the trade recognizes but two colours, *viz.* brown and yellow, the genetical studies carried out reveal the existence of four seed-colours, *viz.* brown, gray, fawn and yellow (Plate LXXI), each being determined by one or more genetical factors.

Every year the hybrids were grown from the selfed seeds of single plants, harvested when fully mature and dried in the sun in thin muslin bags. When fully dry, they were sent to the Imperial Agricultural Chemist who kindly determined the oil-content for us. Oil-content determination was done by the usual ether extract method by means of Soxhlet extractors. Data on oil-content for four years in the case of the first ninety hybrids and for two years in the case of the later-evolved hybrids are available.

The seed-weight and volume were determined by the authors, using thoroughly dried seeds. Dirt and broken seeds were removed. Weight of the seeds was determined by weighing one gram of the seeds and counting the number. This number was taken as an index of the weight of the seed. The seed-volume was determined by noting the volume of alcohol displaced by 500 seeds in a burette. Figures for six years are available for the first ninety hybrids and about three years for the later-evolved hybrids.

Seasonal variation was observed in the oil-content, seed-weight, and volume, but the first does not appear to be as much affected as the other two by this factor. The greatest variation was found in the determinations for the year 1931-32, and hence we have treated these separately and compared them with the average for all years including 1931-32. The studies are not yet complete, but as the findings were so interesting, we considered that their publication might be of some help to others working on this problem. Our results are based only on the hybrids evolved at Pusa, and we cannot say how far they would hold true for other material.

III. CORRELATION BETWEEN OIL-CONTENT AND SEED-COLOUR

It is already pointed out that while in commerce only two colours of linseed, *viz.* brown and yellow, are recognised, four distinct colours are known to geneticists. These are brown, gray, fawn and yellow (Plate LXXI). Each of these is determined by a factor or a set of factors. In crosses between brown and yellow-seeded varieties, the F_1 is brown-seeded, and in the F_2 a ratio of 9 brown : 3 gray : 3 fawn : 1 yellow is obtained. Thus brown colour is double dominant and yellow double recessive, while gray and fawn are single dominants each. The genetics of seed-colour was fully studied by Shaw *et al.* [1931], and it is not necessary to discuss this here.

In our studies we have observed a correlation between seed-colour and oil-content as is evident from the following table in which is shown the average oil-content of hybrids in each of the four seed-colour classes in different years.

TABLE I

Showing the average oil-content of each of the four seed-colour classes in different years

Year	Mean of oil per cent				
	Brown seeds (DG)	Gray seeds (dG)	Fawn seeds (Dg)	Yellow seeds (dg)	General mean
1928-29 . . .	40·04 (24)	40·27 (9)	41·83 (22)	41·28 (19)	41·24
1929-30 . .	39·41 (26)	39·30 (9)	40·50 (27)	40·95 (20)	40·13
1930-31 . .	38·59 (42)	39·42 (11)	40·22 (30)	41·10 (20)	39·64
1931-32 . . .	41·97 (41)	41·88 (11)	43·47 (30)	44·42 (20)	42·88
Mean of each colour class .	40·23	40·26	41·51	41·94	41·00

Standard deviation as based on all the years = 1·5076.

The value of '*n*' for each mean is given in brackets.

The table clearly shows that the mean oil-content of the brown seeds is the lowest and that of the yellow seeds is the highest of all. The gray seeds have a slightly higher mean oil-content than the brown seeds, while the fawn seeds are still higher in oil-content. The figures indicate a negative correlation between the seed-colour intensity and oil-content, *i.e.* the darker the seed-colour, the lower is the oil-content. Analysing the data by the method of 'analysis of variance' as shown in Table II, it is observed that the differences between colours are significant, but the whole of the significant difference is accounted for by the combination (Brown and Gray) *vs.* (Fawn and Yellow). The differences between any other combinations are not significant. This indicates that the factor **G**, which converts yellow seed-colour to gray (**dg**=yellow, **dG**=gray) and the fawn seed-colour to brown (**Dg**=fawn, **DG**=brown) is also responsible for the change in oil-content, *i.e.* it lowers the oil-content. The other factor **D** does not affect oil-content.

TABLE II

Analysis of variance of oil-content in four seed-colour classes in four years

Due to	D. F.	S. S.	M. S.	Ratio S_1^2/S_2^2	Significance
Yellow and Fawn *vs.* Brown and Gray . .	1	189·54	189·54	83·4	Significant
Other colours .	2	8·61	4·305	1·9	Not significant
Years . .	3	616·35	205·45	90·4	Significant
Interaction × years	9	57·18	6·353	2·8	,,
Error . .	345	784·14	2·273		

IV. CORRELATION BETWEEN SEED-SIZE AND SEED-COLOUR

We have shown in the previous chapter that the oil per cent in the seeds of linseed decreases with the increase in the depth of seed-colour, or in other words there is a negative correlation between oil-content and seed-colour intensity. In this chapter we will consider the correlation between seed-size and seed-colour. For this purpose we are presenting Table III in which we have shown the average number of seeds per gram of all hybrids belonging to each of the four seed-colour classes.

TABLE III

Mean size (No. per grm.) of seeds of the four types of colours in different years

Year	Brown seeds (DG)	Gray seeds (dG)	Fawn seeds (Dg)	Yellow seeds (dg)	General mean for each year
1929-30 . .	158·7 (26)	176·0 (9)	172·4 (28)	183·3 (20)	171·1
1930-31 . .	164·4 (29)	177·2 (9)	178·8 (29)	183·1 (20)	174·8
1931-32 . .	148·5 (42)	167·9 (12)	159·4 (30)	168·7 (20)	157·8
1932-33 . .	168·7 (41)	184·6 (11)	183·0 (28)	189·0 (22)	178·7
1933-34 . .	180·7 (42)	196·7 (12)	192·4 (29)	197·7 (22)	189·3
1934-35 . .	179·9 (42)	189·4 (12)	198·5 (30)	206·4 (22)	191·7
General means for seeds of each type of colour . .	167·5	182·4	180·8	188·5	177·6

Standard deviation as based on all the years = 15·36.

The value of '*n*' for each mean is given inside the brackets.

It will be seen from the above table that the brown-seeded hybrids are the least in number per gram, and the yellow-seeded hybrids are the highest in number per gram, *i.e.* they are the heaviest and lightest in weight, respectively. The gray and fawn-seeded hybrids lie between the brown and yellow-seeded hybrids, and they are more or less of the same weight ; both these types of seeds are closer to the seed-weight of yellow-seeded hybrids than to that of the brown-seeded hybrids. We can, therefore, conclude from these figures that the seed-weight increases with the depth of the seed-colour, or in other words, the seed-weight and depth of seed-colour are positively correlated.

Analysing the data again by the 'analysis of variance' method, as shown in Table IV, it is seen that the differences brought about in seed-size by the seed-colours are significant and that the differences in the size of seed in different years are also significant. The data likewise indicate that the seed-size is also controlled by the seed-colour factors, **D** and **G**, the size being the smallest when both the factors are absent and biggest when both are present. When either is present the size is more or less intermediate.

TABLE IV

Analysis of variance of seed-size in four seed-colour classes in six years

Due to	D. F.	S. S.	M. S.	Ratio S_1^2/S_2^2	Significance
Colours . .	3	40682·66	13560·89	57·44	Significant
Years . .	5	74710·40	14942·08	63·29	Significant
Colours and years	15	9858·93	657·26	2·78	Significant
Error . .	563	132917·31	236·09		

V. CORRELATION BETWEEN OIL-CONTENT AND SEED-SIZE

Having established a negative correlation between oil-percentage and depth of seed-colour and a positive correlation between seed-size and depth of seed-colour, one would expect a negative, or at any rate no positive correlation between oil per cent and seed-size. That this is actually so is made evident by calculating the coefficient of correlation, *r*, for these two variables as shown in the following table.

TABLE V

Coefficient of correlation between seed-size (No. per gram) and oil per cent

Size of sample	Year	No. of seeds per gram	Oil per cent	Correlation coefficient *P* (one per cent)
74	1928-29 . .	Data not available	41·24	
82	1929-30 . .	171·1	40·13	0·2830
86	1930-31 . .	174·8	39·64	0·2830
97	1931-32 . .	157·8	42·88	0·2540
91	Average (based on 6 years data for seed-size and 4 years for oil-content)	177·6	41·00	0·2673

It would appear from the above table that oil-content and seed-size are not correlated.

VI. DISCUSSION

That there exists a relationship between oil-content and seed-colour in linseed appears to be unquestionable. The Pusa linseed hybrids with a dark seed-colour have a lesser oil-content than those with a light seed-colour.

Inheritance studies in seed-colour [Shaw *et al.*, 1931] revealed that this character is determined by the factors **M**, **D** and **G**, brown being the double dominant (**MDG**) and yellow the double recessive (**Mdg**) and gray (**MdG**) and fawn (**MDg**) single dominants each. These facts when considered in relation to our findings indicate that the factor **G**, which converts the yellow colour to gray and fawn to brown, lowers the oil-content also.

It is indeed a happy coincidence that high oil-content is found to be correlated with a lighter seed-colour, for such a seed-colour also gives a paler oil which is preferred by the trade to the darker oil obtained from brown seeds. Howard and Khan [1924] observed that the yellow seed was correlated with high oil-content, but they thought this might be so because of the bigger seed-size of yellow-seeded linseeds. That this assumption is probably not correct is suggested by the present study. It appears to be a coincidence that in their study the bold-seeded linseeds happened to be yellow in colour and the small-seeded ones brown.

Considering the seed-colour and seed-size (seed-weight and volume are implied), we observe that these characters bear a relationship with one another such that the darkest seeds (brown) are the boldest and the lightest (yellow) are the smallest. The gray and fawn seeds fall in between these two grades of seed-size. The factors **D** and **G** appear to be responsible for increasing seed-size.

With regard to the relationship between oil-content and seed-weight, considering the above facts, one would expect the two characters to be negatively correlated, or to bear no correlation. Our results bear this out, and contradict the popular belief, that the ' bolder the seed the higher is the oil-content '.

VII. SUMMARY

1. Oil-content in the Pusa linseed hybrids is found to be negatively correlated with the intensity of the seed-colour. Thus dark-seeded hybrids, *viz.* brown and gray, have a lower oil-content than the light-seeded hybrids, *viz.* fawn and yellow. The factor **G**, which converts yellow seed to gray and fawn to brown, appears to be responsible for lowering oil-content.

2. Seed-size (weight and volume) is positively correlated with the seed-colour intensity. The yellow-seeded hybrids are the smallest in weight and the brown-seeded the boldest. The gray and fawn-seeded come between these. The seed-colour factors **D** and **G** appear to increase the seed-size.

3. Oil-content and seed-size are not correlated with each other in these hybrids.

ACKNOWLEDGMENTS

We are indebted to the Imperial Agricultural Chemist and Mr. A. C. Ukil of the Chemistry Section for analysing the oil-content of samples of linseeds from year to year, to Rao Bahadur M. Vaidyanathan, Statistician, Imperial Council of Agricultural Research, and Mr. P. V. Krishna Ayyer, Statistician, Imperial Agricultural Research Institute, for reading through the manuscript and making helpful suggestions, and to Messrs. Hasanain and Vaidya, post-graduate students of this Section, for assistance in the determination of seed-weights and volumes.

REFERENCES

Coleman, D. A. and Fellows, H. C. (1927). *U. S. Dept. Agric. Bull.* **1471**, 1-34.

Dillman, A. C. (1936). *Yearbook of Agriculture, U. S. D. A.*, 745-784.

Eyre, J. V. and Fisher, E. A. (1915). *J. Agric. Sci.* **7**, 120-134.

Howard, G. L. C. and Khan, A. R. (1924). *Mem. Dept. Agric. Ind.* (*Bot. Ser.*) **12**, No. 4, 1-183.

Johnson, I. J. (1932). *J. Amer. Soc. Agron.* **24**, 537-544.

Leather, J. W. (1907). *Mem. Dept. Agric. Ind.* (*Chem. Ser.*) **1**, No. 2, 1-38.

Shaw, F. J. F., Khan, A. R. and Alam, M. (1931). *Ind. J. Agric. Sci.* **1**, 1-57.

Snyder, H. (1905). *Minn. Agric. Expt. Stn. Bull.* **90**, 226-227. (Original not seen).

BREEDING INVESTIGATIONS IN SOME OF THE OLEIFEROUS *BRASSICAE* OF THE PUNJAB

BY
ALI MOHAMMAD, M.Sc. (Agri.), L.Ag.
Botanist for Oilseeds, Punjab Agricultural College, Lyallpur
AND
SAWAN MAL SIKKA, B.Sc. (Agri.), Assoc. I.A.R.I.
Agricultural Assistant, Oilseed Section, Lyallpur

(Received for publication on 6th July 1937)

(With Plate LXXII)

CONTENTS

PAGE.
I. Introduction 849
II. Inheritance of characters 850
  (a) Position of the suture of anther 850
  (b) Hairiness of leaf 852
  (c) Colour of seed 854
  (d) Self-fertility 855
III. Relationship between self-fertility, seed-colour and position of suture of the anthers 858
IV. Newly evolved hybrids 859
V. Summary 860
VI. Acknowledgments 861
  References 861

I. Introduction

In a pevious publication, Ali Mohammad, Singh and Alam [1930] have described the floral mechanism, anthesis and mode of pollination of *toria* (*Brassica napus* L. var. *dichotoma*, Prain) and brown *sarson* (*Brassica campestris* L. var. *sarson*, Prain), together with the results of some of the breeding investigations carried out by them on these two crops. They have shown that the floral mechanism of these crops is such as to reduce the chances of self-pollination to a minimum, and that the flowers consequently depend on some external agency (chiefly insects) for bringing about pollination. Data is presented to show that yields of *toria* are adversely affected if there occur, in November, long spells of cloudy, humid, rainy weather, as the insects do not come out on such days to pollinate the flowers. They also found out that the plants of *toria* and brown *sarson* exhibited a high degree of 'self-incompatibility', and that there occurred a great loss of vigour, when the plants were in-bred (strictly self-fertilized). This led them to conclude that pure lines, as such, have no direct economic utility in these crops, and to obtain improved varieties some sort of group-breeding or 'mass-selection' must be resorted to. In contrast to *toria* and brown *sarson* they have described another form of *sarson* having yellow seeds, the plants of which are highly self-fertile, setting seed freely under bag. They found out that this form of *sarson* crosses readily with both *toria* and brown *sarson*.

Ali Mohammad [1935], as a result of the study of crosses of both *toria* and brown *sarson* with yellow *sarson*, reported that self-fertility behaves as an inherited character independent of the colour of seed, and it was, therefore, possible to obtain self-compatible plants having brown seeds like the common forms of *toria* and brown *sarson*. The practical advantage of these results lay in the fact that the new self-fertile strains would not have to depend on insects for pollination, and consequently, the periodic failure of these crops due to insufficiency of insect visitors would entirely vanish. With this object in view the hybridization between yellow *sarson* and the two forms of oleiferous *Brassicae*, commonly grown in the Punjab, *viz. toria* and brown *sarson* has been continued during the past several years, and some of these crosses have been studied up to F_4. During the course of these investigations, the inheritance of a few morphological characters has been studied, and a large number of new pure-breeding, self-fertile hybrids, having different combinations of characters, have been evolved. The object of the present paper is to present the data, so far collected, on these investigations.

II. INHERITANCE OF CHARACTERS

(*a*) *Position of the suture of anther*

In the flowers of yellow *sarson* the suture of the anthers is situated towards the style (introrse) both in the bud stage and in fully opened flowers. In the flowers of *toria* and brown *sarson*, however, the position of the suture of the anthers is different. In the bud stage the anthers lie with their sutures towards the style, but when the flower begins to open the anthers of the four long stamens of the flower turn half-way round, so that their dehiscing surfaces are turned away from the stigma (extrorse). In F_1 of the crosses yellow *sarson* × *toria* and yellow *sarson* × brown *sarson* the extrorse condition was found to be dominant over introrse. F_2 of these particular crosses, raised in 1934-35, was damaged by frost and no definite ratio of splitting could be established from the small number of plants available. However, the data obtained from the crosses made between *toria* and yellow *sarson* in 1934-35 show that in F_2 (Table I) the extrorse and introrse conditions behaved as simple allelomorphs and segregated according to monohybrid ratio.

TABLE I

Segregation of the extrorse and introrse characters in F_2 in the cross between toria *and yellow* sarson *(1936-37)*

No. of the F_1 plant	Segregation in F_2	
	Extrorse	Introrse
6	105	23
12	156	58
18	91	33
Total observed	352	114
Expected on 3 : 1 ratio	349.5	116.5

$$\frac{\text{Deviation}}{\text{P.E.}} = 0.39.$$ The fit is good.

In F_3 eighty-one introrse and thirty-seven extrorse cultures were studied. All the introrse cultures bred true to this character. Out of the thirty-seven extrorse cultures nine bred true and twenty-eight again splitted like F_1. According to expectations 12.3 true breeding cultures and 24.6 splitting ones should have been obtained.

From the data (Table II) showing the segregation in ten of the splitting cultures in F_3 it is established that the two conditions, representing the position of sutures in yellow *sarson* and *toria* parents, behave as heritable characters, the introrse position being recessive and the difference between the two being monofactorial.

TABLE II

Segregation of extrorse and introrse anthers in F_3 of the cross between toria *and yellow* sarson

No. of the plant in F_2	Position of the suture in F_2	Segregation in F_3		Ratio extrorse to introrse
		Extrorse	Introrse	
3/12	Extrorse	21	6	3.5 : 1
17/15	,,	14	4	3.5 : 1
3/14	,,	24	5	4.8 : 1
3/15	,,	18	9	2 : 1
19/5	,,	18	5	3.6 : 1
9/4	,,	17	14	1.2 : 1
8/12	,,	17	6	2.8 : 1
11/10	,,	9	3	3 : 1
9/5	,,	16	6	2.6 : 1
3/9	,,	26	14	1.8 : 1
Total observed		180	72	
Total expected on 3 : 1 ratio		189	63	

$$\frac{\text{Deviation}}{\text{P.E.}} = 1.9.$$ The fit is good.

(*b*) *Hairiness of leaf*

The plants of the *toria* parent are characterised by glabrous leaves, while the plants of yellow *sarson* and brown *sarson* have small hairs distributed over the upper and lower surfaces of the leaves.

The F_1 plants of the cross yellow *sarson* × *toria* and its reciprocal were hairy, thereby showing the dominance of hairiness over glabrousness.

In F_2 the progenies of only two F_1 plants were studied with regard to the inheritance of hairness and the data obtained (Table III) show that a good fit was not obtained in F_2 according to the expectations.

TABLE III

Segregation of hairiness of leaves in F_2 of the cross between yellow sarson *and* toria

No. of plant in F_1	Segregation in F_2	
	Hairy	Glabrous
6	89	49
12	151	71
Total observed	240	120
Expected on 3 : 1 ratio	270	90

$$\frac{\text{Deviation}}{\text{P. E.}} = 5{\cdot}4.$$ The fit is not good.

The discrepancy may probably be due to wrong classification of the plants, as it is possible, that some really hairy plants may have been classed as smooth. This was actually proved to be so by the results obtained in F_3 as a few cultures, which had been classed as smooth in F_2, were observed to split into hairy and smooth with the former kind of plants predominating.

The data obtained in F_3 and F_4 on the inheritance of hairiness were examined in detail to clarify the whole position. In F_3 the inheritance of hairiness was studied in twenty-five cultures. Out of these, eight cultures came from smooth F_2 parents and each of these bred true to smoothness. Out of the remaining seventeen hairy cultures, five bred true and twelve again splitted into hairy and smooth against an expectation of 5·7 true breeding and 11·3 splitting ones. The actual splitting of nine of these hairy cultures studied in detail in F_3 (Table IV) establishes the monogenic segregation of these characters.

TABLE IV

Segregation of hairiness of leaves in F_3 of the cross between yellow sarson *and* toria

No. of plant in F_2	Character of the F_2 plant	Segregation in F_3	
		Hairy	Glabrous
17-15	Hairy	16	6
17-17	,,	8	5
17-19	,,	7	5
19-5	,,	14	8
21-11	,,	2	1
3-9	,,	29	11
3-14	,,	23	8
3-3	,,	9	1
15-13	,,	8	1
Total observed		116	46
Expected on 3 : 1 ratio		121·5	40·5

$$\frac{\text{Deviation}}{\text{P. E.}} = 1\cdot48. \text{ The fit is good.}$$

The data obtained in F_4 (Table V) regarding the splitting of hairiness in the various cultures further confirm the view expressed above.

TABLE V

Segregation of hairiness in F_4 of the cross between yellow sarson *and* toria

Culture No. in F_3	Character of parent	Segregation in F_4		Ratio of hairy : glabrous
		Hairy	Glabrous	
3-9-52	Hairy	26	12	2.1 : 1
3-14-1	,,	22	6	3.6 : 1
3-14-5	,,	18	4	4.5 : 1
3-14-7	,,	25	8	3.1 : 1
3-14-15	,,	24	6	4.0 : 1
9-4-5	,,	15	6	2.5 : 1
9-4-9	,,	18	7	2.6 : 1
17-15-4	,,	26	8	3.2 : 1
17-15-3	,,	17	5	3.4 : 1
17-15-5	,,	22	6	3.6 : 1
Total oserved		213	68	3·1 : 1
Expected on 3 1 ratio		210·75	70·25	

$$\frac{\text{Deviation}}{\text{P. E.}} = 0\cdot16. \text{ The fit is good.}$$

In conclusion, therefore, it may be stated that hairiness and smoothness of leaves in yellow *sarson* and *toria* respectively behave as simple allelomorphic characters, the former being dominant over the latter and the two segregating in the ratio of 3 : 1.

(c) *Colour of seed*

The colour of seed of the *toria* parent is reddish brown while the yellow *sarson* parent has yellow seeds.

In F_1 the seed colour of the *toria* parent was observed to be dominant. The splitting obtained in F_2 is shown below (Table VI).

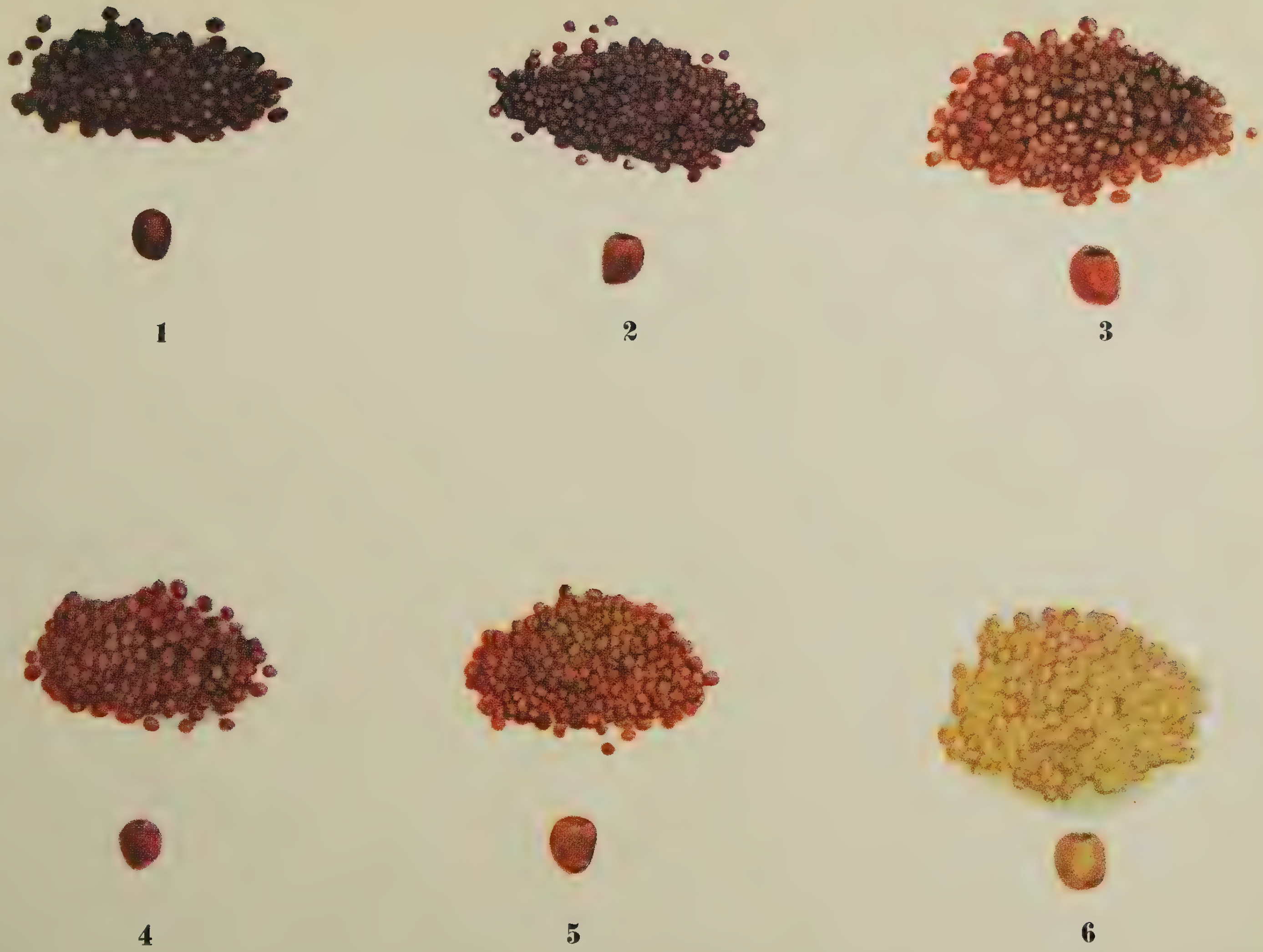
TABLE VI

Segregation of seed colour in F_2 of the cross yellow sarson × toria

No. of plant in F_1	Segregation in F_2						
	Reddish brown	Dark brown	Red	Chocolate	Yellowish brown	Yellow	Total No. of plants in F_2
6	36	33	7	8	6	25	115
12. . . .	100	20	34	..	..	25	179
18. . . .	60	17	20	..	..	13	110

The three F_1 plants, the progeny of which was studied in F_2, behaved differently in the segregation of the seed colour. Plant No. 6 produced six different seed colours in F_2 and in this case the number of yellow-seeded plants is nearly one-fourth of the total number of plants. In the progeny of plant Nos. 12 and 18 the yellowish brown and chocolate classes were non-existant and also the number of yellow-seeded plants is very much less than is the case in plant No. 6. These data evidently suggest that the original parent plants of *toria* (which is highly self-sterile) used in this cross were not genetically pure with regard to the colour of their seed.

Ali Mohammad [1936], as a result of the study of inheritance of seed colour in crosses between *toria*, brown *sarson* and yellow *sarson*, reported as follows :—" The data so far collected regarding the segregation of F_2 progenies show that brown colour is dominant over yellow. The genes for this character segregate in simple Mendelian fashion giving the usual ratio of three dominants to one recessive ". It appears that the material studied by him was of a type similar to that of plant No. 6 reported above in this paper. He, however, did not distinguish



Various seed colours in F_2 of cross *toria* × yellow *sarson*.

1. Dark brown ; 2. Chocolate ; 3. Yellowish brown ; 4. Reddish brown ; 5. Red ; 6. Yellow.

between the various grades of brown colour, as revealed in the present study, and classed them as one on the assumption that the slight differences in seed-colours between the different phenotypes may be due to environmental and nutritional conditions of the plant. In fact, variation in colour of seeds on the same plant does occur as reported by Ali Mohammad, Singh and Alam, but a closer study made in F_4 revealed the fact that many cultures bred true to almost all the colours obtained in F_2 (Plate LXXII).

These findings lead to the conclusion that the reddish brown colour of the *toria* parent is not as simple as thought previously and that more than one factor are responsible for this colour. The segregation obtained from the splitting cultures in F_4 with regard to various seed-colours lends further support to this view. The number of plants in most of these cultures, however, being small, the ratios in which different genotypes split cannot be determined conclusively. Therefore, it is not considered advisable to make an attempt at this stage to suggest any furmula for the factorial constitution of the parents concerned till the seed colour has been studied in greater details. This would now be possible by making further investigations on the material which has become available as a result of the researches carried out so far.

Shaw [1933, 1934] studied the inheritance of seed-colour in crosses between yellow *sarson*, *toria* and brown *sarson*. He obtained results in which the frequency of yellow-seeded plants was much less than the expected number according to 3 : 1 ratio. His material was presumably of a similar nature as the progeny of plant Nos. 12 and 18 reported above. He obtained excess of yellow-seeded phenotypes in one cross (yellow *sarson* 75-1 × *toria* 48-1) while in two other crosses the frequency of yellow-seeded segregates fell below the expected number. Whether the discrepancy in the number of yellow-seeded plants is due to the presence of a lethal factor cannot be stated with certainty at present. It is also possible that the F_2 population being limited, owing to heavy casualties as a result of frost, the chances of realizing the recessive yellow-seeded phenotypes are very much reduced, especially, when the seed colour is controlled by more than one factor, as has been shown to be the case actually. The problems concerned are being further investigated.

(*d*) *Self-fertility*

The plants of *toria* and brown-seeded *sarson* are highly self-sterile. Ali Mohammad, Singh and Alam [1930] found a pod setting under bag of 12.1 per cent and 20·1 per cent on the average results of four years in *toria* and brown-seeded *sarson* respectively. The plants of yellow-seeded *sarson*, however, are self-fertile giving a pod setting under bag of eighty-five per cent or above.

The F_1 of the cross between yellow *sarson* and *toria* was studied during three years (1933-34, 1935-36 and 1936-37), while that of yellow *sarson* × brown *sarson* was studied only in the year 1933-34. The following figures (Table VII) show the percentage of pod-setting under bag in F_1 plants of these crosses.

TABLE VII

Number of F_1 plants with different percentages of pod-setting under bag of crosses between yellow sarson *and* toria *and yellow* sarson *and brown* sarson

Cross	Year	Frequency of pod-setting in F_1 (per cent.)										Total No. of the plants bagged
		0 to 10	11 to 20	21 to 30	31 to 40	41 to 50	51 to 60	61 to 70	71 to 80	81 to 90	91 to 100	
Yellow *sarson* × *toria* .	1933-34	2	3	1	3	...	...	...	...	...	...	9
Toria × yellow *sarson*	,,	2	...	...	2	...	...	3	...	...	1	8
Yellow *sarson* × brown *sarson*.	,,	1	...	...	1	...	2	...	...	...	1	5
Brown *sarson* × yellow *sarson*.	,,	1	...	...	1	...	...	1	...	...	...	3
Toria × yellow *sarson*	1935-36	...	...	...	...	...	...	...	...	...	...	6
		The actual percentage of pod-setting was not counted but three plants showed poor setting under bag and three had fair setting.										
Yellow *sarson* × *toria* .	1936-37	5	4	3	2	1	...	...	3	5	...	23
Toria × yellow *sarson* .	,,	8	...	2	...	...	...	1	1	3	2	17

From this table it is apparent that except in one case (cross yellow *sarson* × *toria*, 1933-34) where all the plants were highly self-sterile, percentage of pod-setting under bag being below forty the F_1 of these crosses showed both kinds of plants, *viz*., self-sterile as well as self-fertile. The occurrence of these two kinds of plants in F_1 of the majority of these crosses is probably due to the impurity of *toria* and brown *sarson* parents with regard to the factors concerned.

It may be noted, however, that the behaviour of the cross yellow *sarson* × *toria* during 1933-34, in which all the plants in F_1 were highly self-sterile, led the senior author [Ali Mohammad, 1935] to conclude that self-sterility was dominant over self-fertility. Unfortunately, the F_2 plants of this cross were severely damaged by frost and no definite ratio of splitting could be ascertained from the study of small number of plants available. But, it was observed that in F_3 all the self-fertile plants selected from F_2 of this cross bred true to self-fertility. No F_2 self-sterile plants, however, could be grown in F_3 to show their segregation The fact that all the F_2 self-fertile plants bred true to self-fertility confirms the recessiveness of this character.

The splitting obtained in F_2 raised from F_1 of the cross *toria* × yellow *sarson* grown in 1935-36 (Table VIII) is again suggestive of the inheritance of self-fertility in the present crosses. It may be pointed out that the two F_1 plants used for these studies gave about fifty per cent pod-setting under bag.

TABLE VIII

Percentage of pod-setting under bag in F_2 *of cross* toria × *yellow* sarson (*1936-37*)

Cross	No. of the F_1 plant	Frequency of pod-setting in F_2 (per cent)										Total No. of plants bagged
		0 to 10	11 to 20	21 to 30	31 to 40	41 to 50	51 to 60	61 to 70	71 to 80	81 to 90	91 to 100	
Toria × yellow *sarson*	12	13	1	4	10	10	13	18	10	8	...	87
	18	8	6	...	3	8	17	16	12	...	...	70

A reference to this table shows that in F_2 the pod-setting of the population under observation ranged between nil and ninety per cent which resembles a segregation generally obtained in case of multiple factors. But if seventy per cent is taken as the lowest limit for self-fertility and thirty per cent as maximum limit for self-sterility, as is generally observed in the case of yellow *sarson* and *toria* parents respectively, then the data (Table IX) approximately approach the monohybrid ratio, *viz.*, 1 self-sterile : 2 intermediate in fertility : 1 self-fertile.

TABLE IX

Segregation regarding self-fertility in F_2 *of cross* toria × *yellow* sarson (*1936-37*)

Plant No. in F_1	Frequency in F_2			Total No. of plants	Deviation / P. E.	Remarks
	Self-sterile	Intermediate in fertility	Self-fertile			
12	18	51	18	87	1·3	The fit is good.
18	14	44	12	70	2·2	The fit is good.
Total	32	95	30	157		
Expected on 1 : 2 : 1 ratio	39·25	78·5	39·25	...	2·5	The fit is good.

The behaviour in F_3 of the various phenotypes in the above cross will be known by growing them next year, but as already quoted above the self-fertile F_2 plants breed true to self-fertility. Therefore, it may be concluded that in the present crosses and within the limits above specified self-sterility behaves as a dominant character and the difference between self-sterility and self-fertility is monofactorial, segregation in F_2 taking place according to 1 : 2 : 1 ratio.

Shaw [1934, 1936] studied the inheritance of self-fertility in crosses of yellow *sarson* with *toria* and black *sarson*. During 1932-33 he classified the plants in F_1 and F_2 into self-fertile and self-sterile assuming that the plants which gave a pod-setting, under bag, of thirty per cent or under were self-sterile and those which gave higher than thirty per cent pod-setting under bag were self-fertile. On this assumption, he concluded that self-fertility was dominant over self-sterility and in F_2 the segregation occurred according to three self-fertile to one self-sterile. No F_3 results have, however, been given by him in further support of these results.

Shaw [1934] obtained results contradictory to those referred to above. From the study of the material grown by him at Pusa he came to the conclusion that the inheritance of self-fertility appears to depend upon multiple factors. But from the material grown by him at the Botanical Sub-station, Karnal, he obtained results similar to those in 1932-33.

The results arrived at by us agree with those of Shaw in so far as they show the monogenic difference between the factors for self-fertility and self-sterility, but differ from him in respect of the dominance of either of these characters in F_1. As already shown above in Table VII, in one case in F_1 of the cross between yellow *sarson* and *toria* all the plants were highly self-sterile while there was not a single case where the whole F_1 was completely self-fertile. These findings coupled with the fact that all the F_2 self-fertile plants maintain their self-fertility in F_3 as observed by us, are definitely in favour of self-fertility being a rescessive character in the particular crosses under reference.

III. RELATIONSHIP BETWEEN SELF-FERTILITY, SEED COLOUR AND POSITION OF SUTURE OF THE ANTHERS

Ali Mohammad, Singh, and Alam [1930] reported that in *sarson* there is a close association between self-fertility and yellowness of colour of seeds. Ali Mohammad [1935], however, observed that self-fertility behaved as an inherited character independent of the colour of seed. In order to obtain more extensive data for coming to a final conclusion the correlations of seed colour and position of suture with self-fertility were determined in F_2 progenies in 1936 (Table X).

TABLE X

Relation of seed colour and position of suture of the anthers with self-fertility

No. of F_2 plant	Colour of the seed	Position of the suture of anthers	Frequencies of pod-setting in F_2 (per cent)										
			0 to 10	11 to 20	21 to 30	31 to 40	41 to 50	51 to 60	61 to 70	71 to 80	81 to 90	91 to 100	Total
6	Brown .	Extrorse .	7	6	3	3	4	14	13	4	1	...	55
		Introrse .	3	...	...	...	1	1	2	1	2	...	10
	Yellow .	Extrorse .	2	1	1	6	1	...	...	3	1	...	15
		Introrse .	...	...	...	...	...	2	...	1	...	...	3
12	Brown .	Extrorse .	6	...	4	3	5	8	12	7	3	...	48
		Introrse .	3	1	...	4	4	...	5	2	3	...	22
	Yellow .	Extrorse .	...	...	...	3	...	3	1	...	2	...	9
		Introrse .	...	...	...	...	...	1	...	1	...	...	2
18	Brown .	Extrorse .	7	4	...	2	6	10	12	3	...	...	44
		Introrse .	1	1	...	1	2	5	1	9	...	...	20
	Yellow .	Extrorse .	...	1	...	...	...	2	2	...	...	...	5
		Introrse .	...	...	...	...	...	...	1	...	...	...	1

These results indicate that there is no correlation between self-fertility, seed colour and the position of the suture. There are, however, very few yellow-seeded plants with introrse anthers showing poor pod-setting under bag, but this may be due to very small number of such plants under observation. The fact that some of the F_3 and F_4 cultures having yellow-seeded plants with introrse anthers were found to be self-sterile lends further support to there being no correlation between any of the factors concerned.

IV. NEWLY EVOLVED HYBRIDS

In view of the findings recorded above, the possibility of evolving new hybrids with different combinations of characters referred to above is evident. As a result of the study of F_4 progenies of these crosses, twenty-five such true-breeding self-fertile hybrids have already been evolved and classified on the basis of the following important morphological characters.

(*a*) *Colour of seed.*—Six different colours of seed, *viz.*, dark brown, reddish brown, chocolate, yellowish brown, red and yellow. Hybrids breeding true for all these colours excepting chocolate have been realized in F_4.

(*b*) Position of the suture of the anthers, which may be extrorse or introrse.

(*c*) Hairs on the leaves.

(*d*) *Time of flowering.*—The new hybrids have been designated as early, intermediate, or late according to the following scale, which has been fixed with due consideration of the flowering time of the parents concerned.

(*i*) Flowering between forty-five and sixty days after sowing............ 'Early'.

(*ii*) Flowering between sixty-one and seventy-five days after sowing ....'Intermediate'.

(*iii*) Flowering seventy-six days or above after sowing ....'Late'.

The economic utility of the above-mentioned new hybrids would depend, primarily, on the yield and percentage of oil in their seeds. Tests with these will be carried out in due course, but from the preliminary observations made on them some appear to have great economic possibilities and may be expected to replace the common forms of *toria* and *sarson* now in cultivation in the province.

V. SUMMARY

1. The common brown-seeded forms of *toria* (*Brassica napus* L. var. *dichotoma*, Prain) and *sarson* (*Brassica campestris* L. var. *sarson*, Prain) are highly self-sterile giving very poor setting under bag and are extensively cross-pollinated in nature, depending almost entirely on insects for pollination. Yellow-seeded form of *sarson*, on the other hand, is self-fertile giving very good setting under bag.

2. The yellow-seeded form of *sarson* has been inter-crossed reciprocally with both *toria* and brown-seeded *sarson* in order to evolve strains which, in addition to possessing all the desirable characteristics of *toria* and brown-seeded *sarson*, should have the invaluable merit of being self-fertile like yellow-seeded *sarson*.

3. In the above-mentioned crosses inheritance of a number of characters (*e.g.*, hairiness of leaves, position of the suture of anthers, seed colour and self-fertility) has been studied up to F_4.

4. Hairiness of leaves is found to be dominant over smoothness, and extrorse condition of the anthers to be dominant over introrse condition. Both these characters are inherited according to simple monohybrid ratio of 3 : 1.

5. The inheritance of seed-colour seems to be complicated mainly because the self-sterile parents cannot breed true. However, the results obtained so far show that the brown colour of the seed of *toria* and *sarson* is dominant over yellow and that more than one factor are responsible for the appearance of various grades of brown colour.

6. The study of the self-sterility is also somewhat complicated owing to the impurity of the self-sterile parents. The scope of a searching study of this character is further limited by the fact that each and every plant has to be bagged for finding out its true nature. However, the results obtained so far show that self-sterility is dominant over self-fertility and the difference between the two is due to a single factor. This conclusion is based on the assumption that plants showing up to thirty per cent pod-setting under bag are treated as self-sterile and those with seventy per cent or more pod-setting are regarded as self-fertile.

7. Data have been presented to show that self-fertility is inherited independently of seed colour and position of the suture of anthers.

8. A number of new self-fertile hybrids having different combinations of characters have been evolved and a key to their identifying characters has been given. Some of these hybrids appear to have great economic possibilities.

VI. Acknowledgments

The results reported in this paper form a part of the programme of work of the five years Oilseed Research Scheme in the Punjab, partly financed by the Imperial Council of Agricultural Research, and we take this opportunity of acknowledging the help received from the above council in the way of providing facilities which have enabled us to make a great progress in the study of many of the important problems connected with the subject.

REFERENCES

Ali Mohammad (1935). *Ind. J. Agric. Sci.* **5**, 1-30.

——————— (1936). " A summary of the more Important Results arrived at or indicated by the Agric. Stations and Res. Officers in the Punjab ", pp. 45-63.

Ali Mohammad ; Singh, R. D. and Alam, Z. (1930). *Ind. J. Agric. Sci.* **1**, 109-131.

Shaw, F. J. F. (1934). *Sci. Repts. Imp. Inst. Agric. Res., Pusa, 1932-33*, pp. 76 & 89-91.

——————— (1936). *Sci. Repts. Imp. Inst. Agric. Res., Pusa, 1933-34*. pp. 77 & 95.

COLOURS IN THE RIÇE GRAIN

BY

K. RAMIAH, M.Sc., Dip. Agri. (Cantab.), L.Ag.
Geneticist and Botanist, Institute of Plant Industry, Indore
(Formerly Paddy Specialist, Coimbatore)

AND

C. RAJASEKHARA MUDALIAR, M.A.
Assistant to Paddy Specialist, Coimbatore

(Received for publication on 16th September 1937)

(With Plates LXXIII-LXXVI)

Introduction

The different colours occurring in the rice grain have received considerable attention regarding their mode of inheritance. The findings of Parnell *et al.* [1917], Hector [1913], Thomstone [1915], McKerral [1913], Van Der Stock [1912], Lien Fang Chao [1928], Ramiah *et al.* [1935], bring out the different aspects of colour inheritance, more especially the segregation of red and white rices. Very little work has been done so far on the anatomical aspect of different rice colours. The only reference that could be cited is that of Junichi Ishikawa *et al.* [1930]. Ishikawa worked on the histological characteristics of red rice, and the most important of his findings are :—

(1) that the seed-coat of the mature grain consists of only one layer of cells and that the colour appears in this layer.

(2) that the first appearance of pigments in the layer is recognisable about three to four days after fertilisation.

(3) that the pigment layer attains maximum thickness about two weeks after fertilisation, then contracts in thickness gradually to 5 to 9 microns about three weeks after.

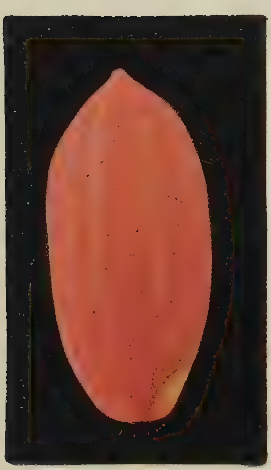
Nilsson-Ehle [1914] made some observations on the histology of red pigmentation in the seed-coats of wheat and found the red pigment in the inner layer of the integument. Percival [1921] records that the rich purple tint observed in certain Abyssinian types is due to the presence of anthocyan pigment located chiefly in the chlorophyll or cross layers of the pericarp.

Preliminary to the histological study of the different rice colours, it is necessary to understand the correct anatomy of the pericarp, as some of the rice colours have been observed to occupy the whole of this region. This anatomy has been studied by several workers previously. According to Vogl [1899] the pericarp layers have been differentiated into the following groups—(1) epidermis, (2) 'Schwamparenchyma', meaning probably the parenchyma of the pericarp, and (3) 'Schlauche' (seed-coat), but he could not observe the true seed-coat. Owaki [1902], however, differentiated five groups made up of (1) epidermis, (2) parenchyma of five to six cell layers, (3) chlorophyll cells of two layers, (4) tube cells, and (5) spermoderm (seed-coat) consisting of three to four layers of cells when young and only one cell layer in the mature grain. Nagai [1910] observed in the ripe grain of rice, only the first four groups of Owaki, but in the milk ripe stage of grain, however, he noticed two distinct cell layers between the tube cells and the aleurone, which he considered must be a part of the integument and the nucellus. He found that the inner layer of the inner integument (spermoderm) which faces the nucellar epidermis was cutinised and thought that it should be the seat of selective permeability in rice. Winton [1932] divides the rice pericarp into six classes (1) epicarp (epidermis), (2) mesocarp (parenchyma of pericarp), (3) cross cells (chlorophyll cells), (4) tube cells, (5) spermoderm (inner integument), and (6) perisperm (epidermis of nucellus).

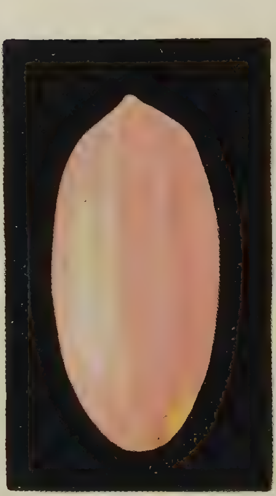
The sections of rice so far examined at Coimbatore, reveal generally the same groupings adopted by Nagai. In the ripe grain, however, the parenchymatous tissue is never ten to twelve cell layers thick as found by Nagai, but consists only of three or four layers of disorganised cells so very compressed that the identity of the cells could not be easily made out. The maximum thickness is to be found in the milk ripe grains, *i.e.*, twelve to fifteen days after fertilisation; the chlorophyll layers are also not to be seen in the section of ripe grain. Perisperm or epidermis of nucellus, has been observed by Schinichi Terada [1928] to lose its cell contents with the formation and development of the endosperm. It is found by the authors that the cell contents persist in many cases until fifteen days after anthesis, after which time disorganisation sets in.

It has generally been observed for most of the rice grain colours examined that the origin and seat of the colouration is the spermoderm or the innermost layer of cells of the inner integument. The existence of the spermoderm in the rice grain is conditioned by the development of pigments. This layer is developed to various degrees depending upon the nature of the colouring matter deposited. In the case of white rices, this layer is completely disorganised by the time the grain matures, and this corresponds with the observations of Junichi Ishikawa [1930].

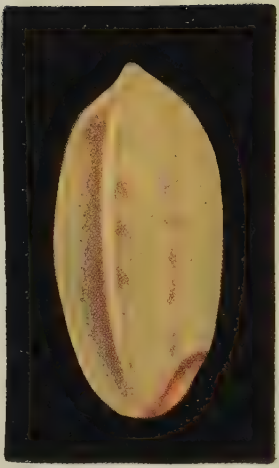
During the investigations on the botanical aspects of 'Quality in rice' financed by the Imperial Council of Agricultural Research, a detailed study of the anatomy of the seed-coat was made. The opportunity afforded by this investigation was taken advantage of in studying the origin and development of different colours occurring in the rice kernel. It will be observed from the references quoted



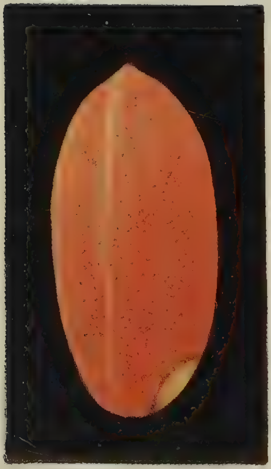
Red



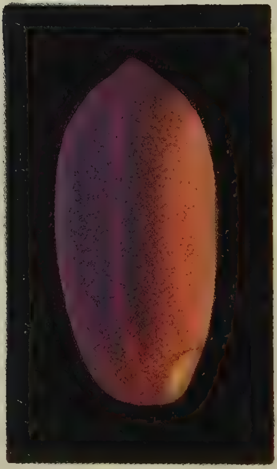
Light red



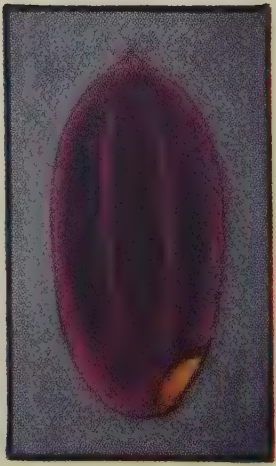
Grey brown



Gold



Brown purple



Dark purple

above that the red colour alone of the rice grain was studied by the different authors while the other colours of mainly genetical interest, occurring in rice, have not been investigated. In this note it is attempted to give a detailed histological account of all the most important colours of the rice grain.

MATERIAL AND METHOD

The rice colours taken up for this study were: red, light red, grey-brown, gold, brown-purple, and dark purple (Plate LXXIII). The following representative types (pure lines) were selected, typical of each colour :—

1. Red rice	T 111 and Co 9
2. Light red	T 356
3. Grey-brown	T 150
4. Gold	T 149
5. Purple	T 134 and T 102

In some preliminary observations made previous to this study, it was found that the rice colour invariably appears after the seventh day after fertilisation, though in the case of dark red rice the pigment might sometimes appear even a day or two earlier. Materials in different stages of development were fixed in formalin acetic alcohol. In the case of red rice, ovaries ranging from the third to the thirtieth day after anthesis were taken. Ovaries up to seven days were passed through the alcohol and xylol series of the usual paraffin method. Ovaries fifteen to thirty days old were treated according to the method adopted by Brenchley [1909] in the case of wheat, and further tried and explained in the case of rice by Rajasekhara Mudaliar [1936]. In this method the grains after fixing and washing are preserved in a mixture of 1/3 glycerine and 2/3 rectified spirit for about a week and then passed on to paraffin infiltration through the medium of bergamot oil. The embedded material is cut and the cut surface soaked in water for at least twelve hours before sections are cut. Longitudinal sections ranging from 10 to 12μ thick were cut and stained in picro-anilin-blue. The colouring matter was not affected by the stain.

OBSERVATIONS

Dark red rices

Two different rices, Co 9 and T 111, in which the kernel gets a deep red colour, were taken up for examination. The colour first appears on the seventh day after anthesis; it is seen lining up the cavity of the spermoderm (Plate LXXIV, fig. 1-*a*), and the colouration is a little more intense on the side of the spermoderm facing the perisperm. With the development of the grain, the colouring matter gradually fills the cavity of the cells (Plate LXXIV, fig. 1-*b*), until in the ripe grain the whole spermoderm and sometimes the pericarp layers are also coloured red (Plate LXXIV, fig. 1-*c*). The thickness of this colouring layer at three different stages of development is given below :—

Variety	Thickness of spermoderm in microns		
	Seventh day	Sixteenth day	Thirtieth day
T 111	6·8	6·9	7·3
Co 9	6·9	11·0	12·3

A perusal of the figures in the above table will show that in the case of T111 the maximum thickness of spermoderm is practically reached even on the seventh day after anthesis, that is, at the time of entry of the pigment in the spermoderm. But in the case of Co 9, another red riced variety, the colouring layer increases gradually in thickness up to the time of maturity. In the case of T 111, the colouration extends to the other pericarp layers also (Plate LXXIV, fig. 1-*c*), but in the case of Co 9 it is restricted to the spermoderm only even at the ripe stage.

Light red rice

In the case of light red colour (Plate LXXIII) the development of the colouring layer is poor when compared to the red rices. The pigment is seen as a very light tinge on the seventh day (Plate LXXIV, fig. 2-*a*) in the inside thick wall of the spermoderm only ; the spermoderm itself is about 4μ thick, but the colour could not be seen in these cells. On the sixteenth day it increases in thickness to about 7μ (Plate LXXIV, fig. 2-*b*), and the light tinge is seen lining the cell cavity of the whole spermoderm, but it is not as distinct as in red rices. The colouring layer is much better developed on the dorsal side than on the ventral. In the ripe grain (Plate LXXIV, fig. 2-*c*) however, the spermoderm is very much reduced in thickness as compared to the sixteenth day, measuring only about 3μ ; but the colour is found to fill the cells completely.

Grey brown rice

T 150 which is a typical grey brown rice was taken for this study. This is an extracted type resulting from a cross between white and red rices. According to Parnell *et al.* [1922] pigmentation factor **A** and red rice factor **R** produce red rice, while the absence of the factor **A** gives rise to grey brown rice. It therefore appears that red, grey brown and gold rices are genetically the same as the factor **R** is present in all of them. No colouring matter can be seen on the seventh day after fertilisation either in the spermoderm or in the inner wall of the spermoderm, and the development of the spermoderm is also very poor (Plate LXXV,

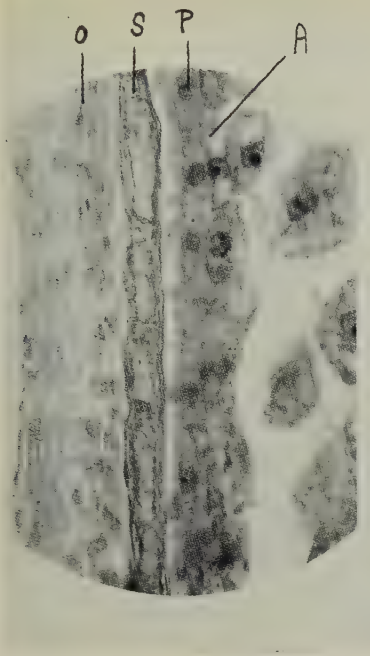


FIG. 1a. Longitudinal section of red rice grain seven days old. Pigment is seen lining the cell cavity of the spermoderm.

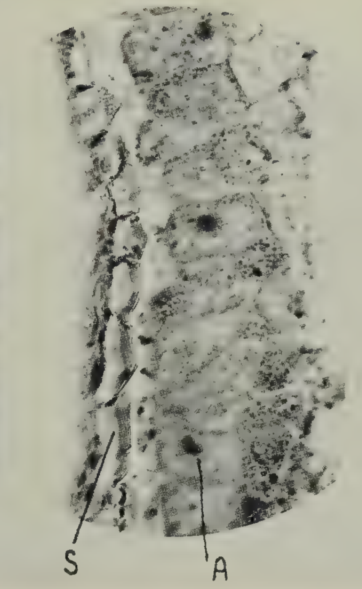


FIG. 1b. Longitudinal section of red rice sixteen days old. Pigment occupies the whole cell.

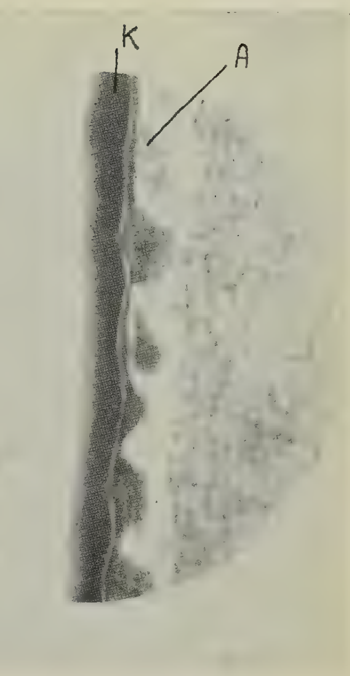


FIG. 1c. Longitudinal section of light red rice seven days old. Pigment occupies the whole pericarp.

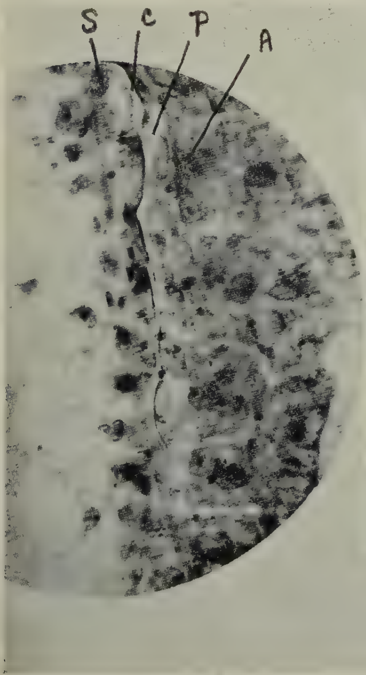


FIG. 2a. Longitudinal section of light red rice seven days old. Colour is restricted to the cutinised inner wall only.

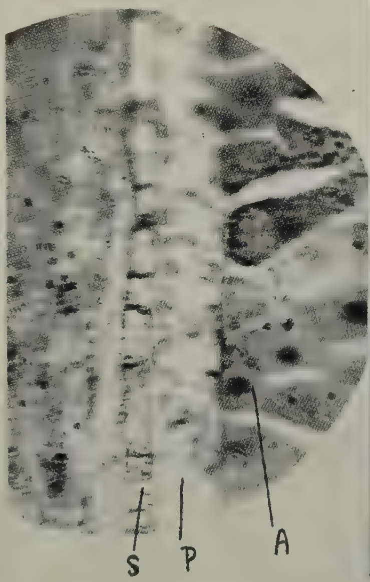


FIG. 2b. Longitudinal section of light red rice, sixteen days old. Light red tint is seen in the spermoderm.

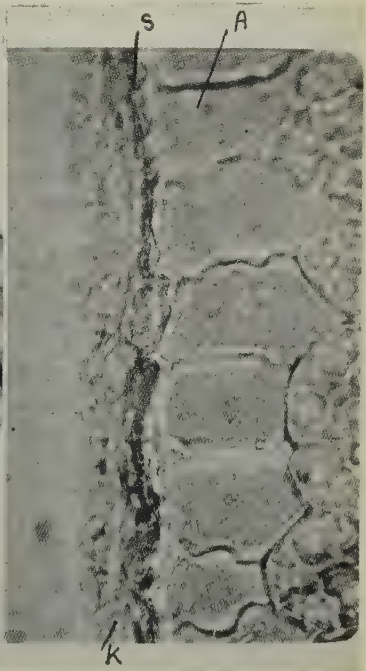


FIG. 2c. Longitudinal section of light red rice thirty days old. The colouring layer is very much reduced in size.

O—Ovary wall ; S—Spermoderm ; P—Perisperm ; A—Aleurone ; K—Pericarp ; C—Cutinised inner wall. Magnification (×750) approximately.

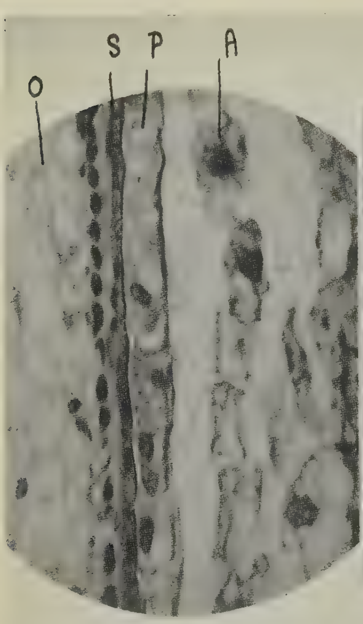


FIG. 1a. Longitudinal section of grey brown rice seven days old. Pigment could not be seen anywhere.

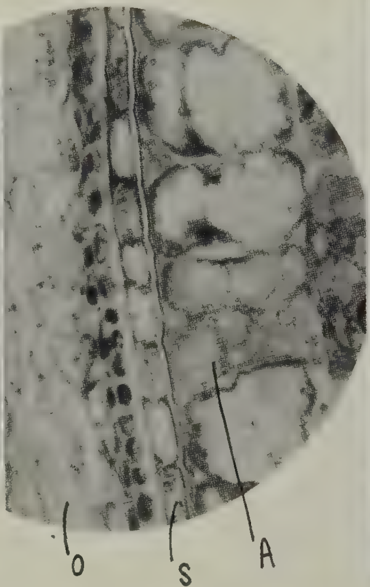


FIG. 1b. Longitudinal section of grey brown rice sixteen days old. Pigment is seen in the spermoderm.

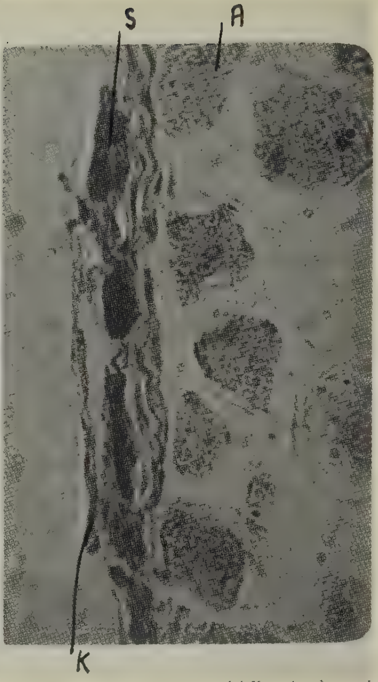


FIG. 1c. Grey brown rice thirty days old. Colour occurs as globules in occasional cells of the spermoderm.

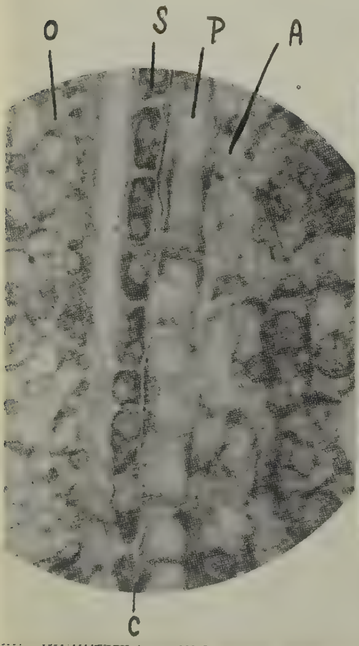


FIG. 2a. Section of gold rice seven days old. Colour is seen faintly in the cutinised wall.

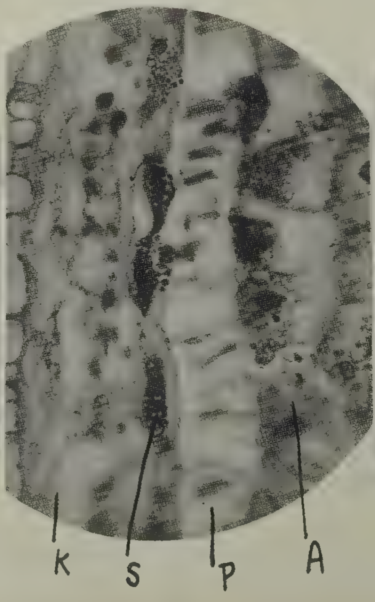


FIG. 2b. Section of gold rice sixteen days old. Colour is seen as globules in the spermoderm.

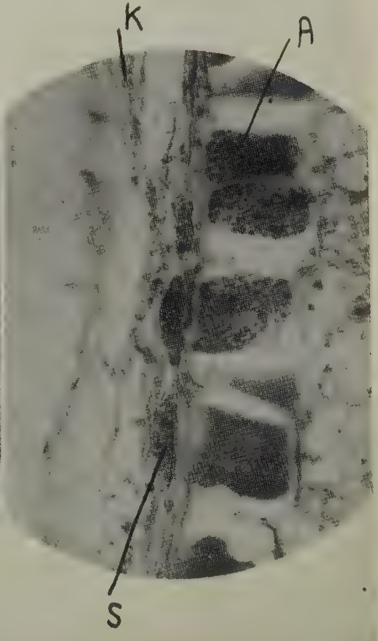


FIG. 2c. Section of gold rice 30 days old. Spermoderm bulged out.

O—Ovary wall ; S—Spermoderm ; P—Perisperm ; A—Aleuzone ; K—Pericarp ; C—Cutinised fig. 1-*a*). On the sixteenth day the colouring matter is seen in the seed-coat (Plate LXXV, fig. 1-*b*) ; it is much deeper in the inner wall than inside the cell. At the ripe stage (Plate LXXV, fig. 1-*c*) the colouring matter is found to be in globules, unlike the red rices in which the pigment is uniformly distributed. The thickness of the colouring layer is given below :—

Variety	Thickness in microns			
	Sixteenth day		Thirtieth day	
	Dorsal	Ventral	Dorsal	Ventral
T 150 (Grey brown rice)	7·6	6·2	5·8	2·9

It will be seen that in the dorsal side the layer is thicker than in the ventral side and this difference is more marked in the ripe grain than in the milk ripe stage. It may also be noted that as the grain ripens, the cells contract and the thickness gets reduced.

Gold rice

Gold colour of rice is distinct from brown (Plate LXXIII). The gold rice [Parnel *et al.*, 1922] was obtained as an extracted type from a cross between red rice and white rice, the gold rice resulting from a factor **R** (red rice) in the presence of factor **I** (gold coloured lemma and palea). On the seventh day after fertilisation the pigment is slightly seen in the inner wall of the spermoderm only (Plate LXXV, fig. 2-*a*). The spermoderm is fairly well developed though the colouring matter has not actually entered the cells ; the thickness of the seed-coat at this stage is only 6μ. On the sixteenth day after anthesis the pigment is seen as globules in some of the cells of the seed-coat (Plate LXXV, fig. 2-*b*), and as the grain ripens the colouring matter gradually fills the cell cavity. The spermoderm measures about 10μ on the sixteenth day, but at the ripening time it measures about 4μ only. In the ripe grain the spermoderm is not uniformly thickened as in the case of red rices but occasional cells here and there get bulged out probably as a result of the entry of the pigments (Plate LXXV, fig. 2-*c*). In this respect it resembles the grey brown rice to a certain extent.

Purple rice

Purple rices may be classed into two groups, those having a dark purple (almost black) pericarp and those having streaks of brown colour known as brown purple rices (Plate LXXIII). In the first, the pericarp, is completely purple while in the second a brownish tinge is developed particularly on the side at the lower end of the grain. The latter type is an extracted pure line from the cross between dark purple and white rice. Though the F_2 segregation of this cross gave a simple 3 : 1 ratio of purple to white the purple groups showed considerable variations ranging from dark purple to a type where the purple is very considerably reduced and a definite brownish tinge appears. In the subsequent generations the very dark colour of the parent was rarely reproduced, the majority being rather lighter and slightly brownish and some of these lighter types were found to breed true.

In the initial stages of grain development there seems to be no difference between these two types of colour on the grain. The pigment is confined to the inner cutinised wall of the spermoderm only, on the seventh day after anthesis (Plate LXXVI, fig. 1-*a*) ; at this stage the spermoderm cells do not show the colour and measure about 8μ in thickness. On the sixteenth day, however, the whole pericarp is full of the purple pigment (Plate LXXVI, fig. 1-*b*). The spermoderm cells are disorganised and cannot be identified with the rapid development of the pericarp cells consequent on the entry of the colouring matter. In the case of the brown purple rice, the purple colour on the ventral side fades gradually towards the embryo, and the pericarp layers immediately surrounding the embryo have very little purple tint. Similarly on the dorsal side of the grain also the colour fades towards the base (morphological top). At the ripe stage the disposition of the colouring matter just resembles, in general, the stage recorded for the sixteenth day (Plate LXXVI, fig. 1-*c*) ; but in the pericarp layers just near the embryo brownish pigment is more profusely deposited. On the dorsal side, the purple pigment is only sparsely deposited towards the base, but in the other portions of the grain the colouring matter is full ; but occasionally brownish spots could also be seen. In the dark purple rices the formation of colouring matter closely resembles the brown purple rice, the only difference being the absence of brownish tint. The pigment on the ventral side is also lighter in shade than on the dorsal. The thickness of the pericarp which is the colouring layer in purple rices is given below :—

Variety	Thickness of pericarp in microns			
	Sixteenth day		Thirtieth day	
	Dorsal	Ventral	Dorsal	Ventral
T 134, Brown purple .	115	52	58	38
T 102, Dark purple .	102	29	35	18

On account of the deposition of the colouring matter in the pericarp layers, this portion of the rice grain is very well developed in these varieties. Though the thickness gets reduced at the time of maturity, it is much thicker than the pericarp layers of white rices at the ripe stage.

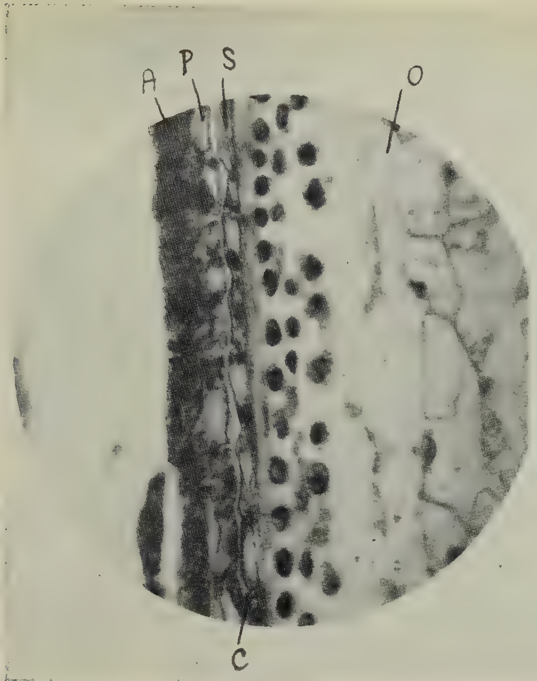


Fig. 1a. Section of purple rice seven days old. Pigment is seen only in the cutinised wall.

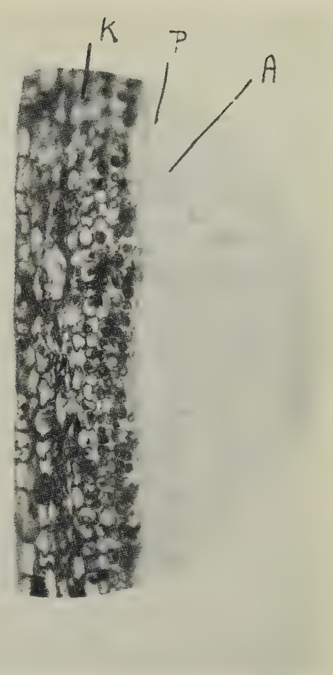


Fig. 1b. Section of purple rice sixteen days old. Pigment occupies the whole of the pericarp. Spermoderm cannot be identified.

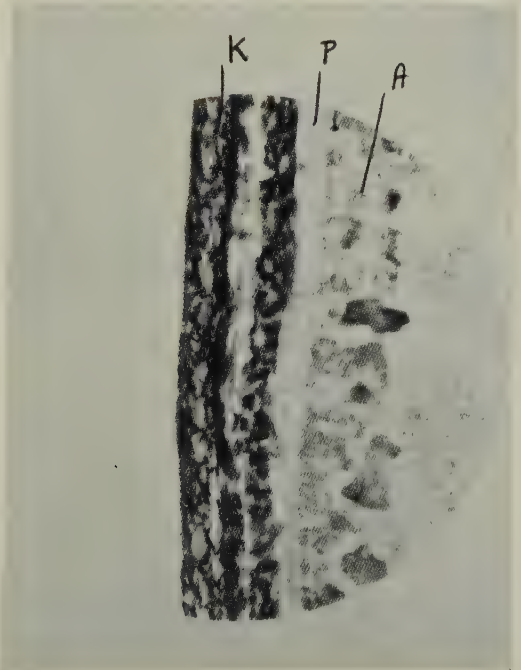


Fig. 1c. Section of purple rice thirty days old. Pericarp thickness is reduced a little. Perisperm still persists as empty cells, a peculiarity among the glutinous rices.

O—Ovary wall ; S—Spermoderm ; P—Perisperm ; A—Aleurone ; K—Pericarp ; C—Cutinised inner wall. Magnification (×750) approximately.

DISCUSSION

It could be seen from the observations recorded above that only in the case of dark rice the colouring matter is deposited in the early developmental stages, directly in the cells of the spermoderm; while in the other coloured rices, *i.e.*, light red, grey brown, gold and purple rices—the colouring matter first appears in the cutinised wall of the spermoderm and later on spreads to the interior of the cells. It will thus be seen that the seed-coat in one way or the other is the seat of colouration in the rice grain. While the findings of Junichi Ishikawa in the case of red rices, and Nilsson-Ehle in the case of wheat have been confirmed, the investigations here recorded are extended to some of the other important colours occurring in rice that have not been studied before. Junichi Ishikawa observed in the case of red rice that the maximum thickness of the spermoderm is attained about two weeks after fertilisation and then the thickness is gradually reduced to five to nine microns. In the case of the two red rices dealt with in this note, it is seen that in one variety the maximum thickness was reached even on the day of the entry of the pigment (seventh day), while in the second it was attained about the fifteenth day ; it appears therefore that varietal differences affect to a certain extent the nature and development of pigments even in one particular colour.

In the thickening of the occasional spermoderm cells, the grey brown and the gold rices resemble one another though the nature of the pigments deposited may vary. In the light red rices, though the pigment is uniformly deposited, the poor development of the spermoderm cells is a characteristic feature.

Purple colour in rice appears to be quite distinct from the other colours. In purple rices the spermoderm which is so conspicuous in other coloured rices, probably plays very little part. Traces of purple pigments are observed in the initial stages of development (seventh day) in the thicker inner wall of the spermoderm only, while the entire body of the cells are free from any pigment. In the later stages of development this layer is, however, disorganised, while at the same time copious quantities of colouring matter are deposited in the whole of the pericarp region. The findings of Percival [1921] " that the rich purple of certain Abyssinian wheat is due to the presence of the pigments in the chlorophyll and cross layers of the pericarp " correspond in a way to the nature of the pigments deposited in the purple and in some of the red rices (T 111) recorded here. It is quite probable that even in the purple wheat, the pigment first originates from the spermoderm and later spreads to the other pericarp layers. In purple rices it is the pericarp layers that play a more important part than the spermoderm. There appears to be no particular difference in the anatomical structure of the colouring cells between the dark and brown purple rices. Traces of brown pigments deposited along the ventral margin of the grain and more especially near the embryo, appear to impart to this variety the characteristic brownish streak on a background of dark purple.

It is not known what role these different colours in rice play in the germination and further development of the embryo. Nilsson-Ehle [1914] observes in the case of wheat that the red grains germinate slower than the white ; and again Nagai [1910] observes that water absorption is slightly affected in the red pericarp colour though the germinating power remains unaffected. The same author observes that in the case of maize, the presence of pigments in the pericarp do not affect the absorbing power of water. The role played by the different colours in the absorption and germination of rice is under investigation at Coimbatore. Nagai finds that the red colour is not affected by acid, alkali, or alcohol ; in the present study it has been found that Nagai's observations are confirmed in the case of red, light red, grey brown and gold rices. But in the purple rice, the pigment is easily drawn out by acids—such as acetic acid—used in fixing and the alcohols, but only a portion of the pigment is thus dissolved. Part of the pigment in the purple rices is also water-soluble since the water in which it is soaked gets a colouring like a weak permanganate solution after some hours. It is quite probable that the colours other than purple being deep-seated in the cells of the spermoderm remain unaffected by these reagents while the purple colour occupying the whole pericarp region is easily attacked. Even in the case of one of the red rices examined (T 111), in which the red colour occupies the whole of the pericarp, the colour is not attacked by these reagents.

SUMMARY

(1) In dark red rices, the colour is noticed in the cells of the spermoderm on the seventh day after anthesis. These cells attain maximum thickness even on the seventh day in some cases, while in certain others, the maximum thickness is reached on the fifteenth day only. The spermoderm cells are uniformly thickened and the deposition of the colour is also uniform.

(2) In light red rices the pigment can be seen only along the inner cutinised wall of the spermoderm on the seventh day and extends to the cell cavity on the sixteenth day. By the time the grains ripen to maturity the spermoderm cells are very much reduced in thickness to about 3μ from 7μ.

(3) The pigment cannot be seen anywhere in the spermoderm in grey brown rices on the seventh day after anthesis. On the sixteenth day the spermoderm cells measure 7μ and the pigment also occurs as globules in the cell cavity, as distinct from the red rices in which the colouring is uniformly distributed. As the grain ripens, these cells are reduced in thickness.

(4) In purple rices the colour is confined to the cutinised wall only on the seventh day. The colour is seen occupying the whole pericarp region by the sixteenth day, when the spermoderm is also disorganised. The colouring layers which are the pericarp cells in these varieties, reach a maximum thickness of 100μ to 115μ at this stage. In the ripe grain, however, the thickness of this layer is reduced

to about 35μ to 58μ. As regards the general disposition of the colour, the dark purple and brown purple rices are alike ; in the latter class, however, in portions of the dorsal and ventral side, more especially near the embryo, the purple colour is lighter, and more of brownish pigments can be seen.

ACKNOWLEDGMENTS

We are indebted to the Imperial Council of Agricultural Research for providing funds to carry on this work as part of the scheme of investigations on ' Quality in Rice '.

REFERENCES

Brenchley (1909). *Ann. Bot.* **23,** 117.

Hector, G. P. (1913). *Mem. Dept. Agric. Ind.* (*Bot. Ser.*) **6,** 8.

Junichi Ishikawa and others (1930). *J. Soc. Trop. Agric.* **2,** 165.

Lien Fang Chao (1928). *Genetics* **13.**

McKerral (1913). *Agric. J. Ind.* **8,** 326.

Nagai (1910). *J. Col. Agric. Imp. Univ.* (*Tokyo*) **3,** 117.

Nilsson-Ehle (1914). *Pflanzenzuchtung.*

Owaki (1902). *Saikin Beikoku Ron.*

Parnell, F. R. ; Rangaswami Ayyangar, G. N. and Ramiah K. (1917). *Mem. Dept. Agric. Ind.* (*Bot. Ser.*) **9.** No. 2.

Parnell, F. R. ; Rangaswami Ayyangar, G. N.; Ramiah K. and Srinivasa Ayyangar, C. R. (1922). *Mem. Dept. Agric. Ind.* (*Bot. Ser.*) **11.** No. 8.

Percival (1921). ' The Wheat Plant ', London.

Rajasekhara Mudaliar, C. (1936). *Proc. Assoc. Econ. Biol.* (*in press*).

Ramiah, K. and Rajasekhara Mudaliar, C. (1935). *Mad. Agric. J.* **23.**

Thompstone (1915). *Agric. J. Ind.* **10,** 45.

Van Der Stock (1912). *Die Zuchtung der Landw.*

Vogl. (1899). *Die Wichtigsten ..... genussmittel.*

Winton (1932). ' Structure and Composition of Foods '.

A NOTE ON TWO NEW GENES AFFECTING ANTHOCYANIN PIGMENTATION IN ASIATIC COTTONS

BY

J. B. HUTCHINSON

AND

R. L. M. GHOSE

Institute of Plant Industry, Indore, Central India

(Received for publication on 27th August 1937)

I. Red spotless

The inheritance of anthocyanin pigmentation in Asiatic cottons has been studied by Hutchinson [1932]. He described a multiple allelomorph series of six members controlling anthocyanin distribution. In a later paper [Hutchinson, 1934] he drew attention to the serial relationship between the allelomorphs, each gene determining the development of anthocyanin in one more area than the member below it, and interpreted the sequence in terms of his theory of the organisation of the gene.

In 1934, seed of a red-leaved form of *G. arboreum* var. *neglectum** was received from China. Anthocyanin developed strongly in stems, leaves, calyx, and bolls, but not in either the lobe or the base of the petal. The distribution differed from that of the $\mathbf{R}_2^L$ allelomorph in the absence of the spot at the base of the petal. It will be shown below that the Red spotless of the Chinese strain is an allelomorph in the anthocyanin series. The symbol $\mathbf{R}_2^O$ may be adopted for it. Below is given in tabular form a summary of the anthocyanin distribution in the allelomorphs so far known.

Gene	Plant organ				
	Petal lobe	Leaf	Calyx	Spot	Basal
$\mathbf{R}_2^R$	+	+	+	+	+
$\mathbf{R}_2^L$	—	+	+	+	+
$\mathbf{R}_2^O$	—	+	+	—	+
$\mathbf{R}_2^C$	—	—	+	+	+
$\mathbf{R}_2^S$	—	—	—	+	+
$\mathbf{r}_2^o$	—	—	—	—	+
$\mathbf{r}_2^g$	—	—	—	(+)	—

---

* Specific and varietal names are as interpreted by Hutchinson and Ghose [1937].

The gene nomenclature used is that of Hutchinson and Silow [1937].

Red spotless was crossed with types carrying $\mathbf{R}_2^{R}$, $\mathbf{R}_2^{L}$, $\mathbf{R}_2^{S}$, and $\mathbf{r}_2^{g}$.

Red spotless × *1056* ($\mathbf{R}_2^{O} \times \mathbf{R}_2^{R}$)

F_1 was $\mathbf{R}_2^{R}$.

In F_2 twenty-seven families give a total of 686 $\mathbf{R}_2^{R}$: 229 $\mathbf{R}_2^{O}$, almost exactly 3 : 1.

Red spotless × *H 9* ($\mathbf{R}_2^{O} \times \mathbf{R}_2^{L}$)

F_1 was $\mathbf{R}_2^{L}$.

In F_2 eight small families gave 31 $\mathbf{R}_2^{L}$: 11 $\mathbf{R}_2^{O}$, again almost exactly 3 : 1.

Red spotless × *Malvi 9 and Red spotless* × *Kokati narrow* ($\mathbf{R}_2^{O} \times \mathbf{R}_2^{S}$)

F_1 was phenotypically $\mathbf{R}_2^{L}$.

In F_2 three classes appeared, $\mathbf{R}_2^{O}$ and $\mathbf{R}_2^{S}$, the parental classes, and a red-leaved, spotted class, phenotypically $\mathbf{R}_2^{L}$, like the F_1. In Red spotless × Malvi 9 F_2, twenty-six families gave 367 $\mathbf{R}_2^{O}$: 826 $\mathbf{R}_2^{L}$: 368 $\mathbf{R}_2^{S}$. For the deviations from 1 : 2 : 1, $\chi^2 = 5 \cdot 3$, $n = 2$, P between 0·1 and 0·05. In Red spotless × Kokati narrow, thirty-nine families gave 907 $\mathbf{R}_2^{O}$: 1730 $\mathbf{R}_2^{L}$: 828 $\mathbf{R}_2^{S}$. For deviations from 1 : 2 : 1, $\chi^2 = 3 \cdot 6$, $n = 2$, $P = 0 \cdot 2$.

Red spotless × *N 6* ($\mathbf{R}_2^{O} \times \mathbf{r}_2^{g}$)

The F_1 plants were red-leaved, with a petal spot surrounded by a clear white margin $\left(\frac{\text{spot}}{\text{ghost}}\right.$ of Hutchinson [1932]$\left.\right)$. In F_2 three classes appeared, the two parental classes, $\mathbf{R}_2^{O}$ and $\mathbf{r}_2^{g}$ and the F_1, $\mathbf{R}_2^{S/G}$. Sixteen families gave 206 $\mathbf{R}_2^{O}$: 504 $\mathbf{R}_2^{S/G}$: 247 $\mathbf{r}_2^{g}$. χ^2 for deviations from 1 : 2 : 1 = 6·0, $n = 2$; $P = 0 \cdot 05$.

Although considerable deviations from expected 1 : 2 : 1 ratios occurred in several cases, the fact that $\mathbf{R}_2^{O}$ is a member of the allelomorph series is proven beyond doubt. The deviations were not consistently in one direction, and require no special consideration.

The discovery of an allelomorph which does not fit into the series of step by step reductions in anthocyanin distribution necessitates a revision of Hutchinson's [1934] speculation on the organisation of the anthocyanin genes. It becomes necessary to postulate more gene centres. If one gene exists in which the order of reduction is changed, others may also, and all combinations of anthocyanin distribution may ultimately be found.

II. SMALL SPOT

A strain of *G. arboreum* var. *neglectum* with a very small petal spot was received from the Economic Botanist (Cotton), Nagpur. It was grown under the number 1079. The spot in 1079 varied from about one-eighth the size of the normal spot to a few pigmented cells only.

1079 was crossed to a Full spot ($\mathbf{R}_2^{\mathrm{S}}$) strain, and to Red spotless ($\mathbf{R}_2^{\mathrm{O}}$).

1079 × Full spot

Small spot was dominant in F_1. In F_2, an attempt was made to classify spot size against a series of ink drawings differing in size only. Grade 1 was a very small spot and grade 7 Full spot. The attempt was unsuccessful, owing to variations in the size of the petal, which affected the size of the spot, and to the fact that no allowance was made for differences in intensity of pigmentation. Twelve F_3 families were grown, and were classified by eye on size relative to petal size, and intensity of spot. They fell clearly into three groups, Small, Intermediate, and Large. F_3 data are summarised in Table I below.

TABLE I

Spot segregation in F_2 of 1079 × Full spot

Parental grade	Observed			Expected		
	Small	Intermediate	Full	Small	Intermediate	Full
7	..	..	46		homozygous Full	
7	..	..	45			
7	..	1	63			
7	..	..	46			
5	..	..	45			
7	..	..	95			
6	2	34	12	..	36	12
6	..	32	12	..	33	11
2	52	19	24	54	18	24
2	44	12	19	42	14	19
1	46	5	..?	39	13	..
1	40	1	..		homozygous Small	

Expected ratios are given on the supposition that two factors are involved, a spot reducer, **Sr**—**sr**, reducing Full spot to Intermediate, and a second factor, which reduces Intermediate spot **Sr** to Small spot, giving a 9 : 3 : 4 ratio in F_2. The existence of the main spot reducer is clearly demonstrated, but the behaviour of the two progenies from grade 1 parents makes it impossible to rely with confidence on the distinction between Small and Intermediate.

1079 × Red spotless

The F_1 plants were Red small spot.

In F_2 seven classes appeared, Red spotless, Red small, Intermediate and Full spot, and Green small, Intermediate and Full spot. F_2 data are given below.

R_2^O	R_2^L			R_2^S			
	Small	Inter.	Full	Small	Inter.	Full	Total
131	114	47	65	48	41	37	483
	226			126			

The main anthocyanin segregation gave a close fit to 1 : 2 : 1. The main spot reducer segregation was also satisfactory.

	Small and Inter. (**Sr**)	Full (**sr**)	Total
R_2^L	161	65	226
R_2^S	89	37	126
Total	250	102	352

χ^2 (3 : 1)=2·97, $n = 1$, $P = 0{\cdot}1$.

The segregation into Small and Intermediate, however, was not simple, and the genetic basis of the distinction must be left in doubt pending further work. The apparent association of Small spot with the heterozygous R_2^L class is probably due to intensification of the effect of **Sr** when the main spot gene is heterozygous.

III. Summary

A new member, Red spotless, R_2^O, of the anthocyanin multiple allelomorph series is described, and its position in the series established. Red spotless differs from Red leaf, R_2^L, in the absence of petal spot. It is pointed out that the discovery of this gene necessitates reconsideration of Hutchinson's [1934] speculation on the organisation of the anthocyanin genes, and leads to the expectation that genes may be discovered giving further combinations of anthocyanin distribution.

The existence is demonstrated of a petal spot reducer, **Sr**, in a strain of *G. arboreum* var. *neglectum* from Nagpur. A second factor probably operates to intensify the effect of **Sr**, but it has not yet been possible to analyse its mode of action completely.

REFERENCES

Hutchinson, J. B. (1932). *J. Genet.* **26**, 317.

—————— (1934). *J. Genet.* **28**, 437.

Hutchinson, J. B. and Ghose, R. L. M. (1937). *Ind. J. Agric. Sci.* **7**, 233.

Hutchinson, J. B. and Silow, R. A. (*In press*).

EFFECT OF FARMYARD MANURE ON FIBRE-CHARACTERS OF COTTON

BY

H. R. NAYAK

Technological Assistant, Dharwar

(Received for publication on 4th November 1936)

(With seven text-figures)

INTRODUCTION

CONFLICTING statements with regard to the effect of manuring on the fibre-characters of cotton occur in literature. All-extensive study, principally aimed at elucidating this point, does not seem to have been so far seriously taken up anywhere. An opportunity to analyse the cotton from the point of view of fibre-characters and raised in plots receiving varying rates of farmyard manure, presented itself to the writer at the Government Experimental Farm, Dharwar, and the results obtained are reported in this paper.

LITERATURE REVIEWED

Balls [1915] concluded that the conditions which modified the physiological functions of a plant might affect the length and strength of fibre. Certain irrigation studies by him revealed for instance that a deficiency in soil moisture for a period of twenty-three days, soon after flowering, was associated with a shortening of lint-length.

Youngblood [1929], in a study of correlation between reported lint-length of cotton and the nature of the soils where it was grown, reached the conclusion that longer lint was associated with more fertile soils but in the case of the poor soils of Piedmont, longer lint than on the more fertile soils of coastal plains was produced.

Hawkins and Serviss [1930] thought that the prevailing temperatures contribute to the rate of fibre development and, when lower than necessary for optimum plant growth, have a retarding effect on both fibre elongation and fibre-wall thickening.

Armstrong and Bennett [1933] found no significant difference in lint-length of plants raised on poor soils and plants of the same variety grown on high fertility soils. They noted however that bolls resulting from blooms that were among the last to be retained, were 1/16 to 1/8 inch shorter than those originating a week earlier. They also noted that bolls from blooms opening on the same date and with a long maturation period had a larger percentage of short hairs than those with a short maturation period.

Reynolds and Killough [1933] using the maximum length of combed hairs removed from the seed as a length measure found no significant correlation between fibre-length and amount of either nitrogen, phosphoric acid, potash or rates of application of fertiliser in Central Texas. In Eastern Texas, where in sandy soils phosphoric acid is deficient, significant increase in length of fibre was obtained on its application.

Sturkie [1934] concluded that length of lint was markedly affected by soil moisture. Favourable soil-moisture conditions produced heavy bolls with long lint, heavy seed, large lint index and low percentage of lint.

Nazir Ahmad and Sen [1934] found that the application of fertilisers either failed to produce any significant increase in yield or even caused a depressing effect where the soil was naturally rich. But in soils poor and deficient in substances necessary for plant nutrition, a heavy increase in yield was obtained by using fertilisers. The results of the fibre and spinning tests showed that the fertiliser treatment resulted in somewhat stronger and less neppy yarns if the soil fertility was originally low, but that no such improvement was observed or even a negative effect may be produced where the soil was inherently rich.

Gracie and Fanny Khalil [1935] investigated the influence of factors such as variety, water supply, spacing, soil, climate, etc. in governing the response to manuring of cotton in Egypt on a sufficiently large scale and opined that yield effects from nitrogen are positively correlated with high level of yield and increased proportion of crop at the second picking.

Pope [1935] concluded that the higher rates of application contribute to an increased seed weight and weight of seed cotton. The weight of lint per 100 seeds showed no definite trend which might be considered due to the rate of application. Percentage lint tended to decrease with increasing rate of fertiliser, this being due to the relatively stable amount of lint per seed and a seed weight which increased with amount of fertiliser application. The data obtained by Pope [1935] suggest that a slight increase in length may be associated with the higher rates of application. The data on tensile strength indicated that a decreasing strength accompanied an increasing rate of application which was in general agreement with other findings which indicated that a decrease in strength was usually associated with more favourable growth conditions.

MATERIALS AND METHODS

Jaywant cotton was selected for this study. It is a hybrid of two pure-line selections belonging to *Gossypium herbaceum* L. and possesses good characters of both parents, namely, staple and resistance to *Fusarium* wilt. The cotton was grown at the Government Experimental Station at Dharwar and the samples were kindly supplied by the Farm Superintendent.

The experiment was laid out in duplicate plots, each measuring four *guntas* or one-tenth of an acre. So there were in all twelve plots, six for each of the original and duplicate series. The plots were arranged in such a way that the plot numbers 1, 2, 3, 4, 5 and 6 which received 2 1/2 tons to cotton, 2½ tons to *jowar*, five tons to *jowar*, ten tons to *jowar*, no manure and fifteen tons to *jowar*, all per acre, respectively, were next to one another. Immediately next to these six plots, duplicate plot numbers 6, 5, 4, 3, 2 and 1 which received fifteen tons to *jowar*, no manure, ten tons to *jowar*, five tons to *jowar*, 2½ tons to *jowar* and 2½ tons to cotton, all per acre, respectively followed one another in succession and in a line with the original plots. The layout of the farmyard manure experiment, showing the position, size and the various treatments given to the plots is depicted in Fig. 1.

The material for the study was obtained from an established test in which farmyard manure was applied in increment steps of 2½ tons ranging from none to fifteen tons per acre. In each case a systematic planting plan was used. In many cases, the manure was not given directly to cotton crop, but to *jowar*, which was grown previous to cotton. Samples of about three pounds of *kapas* were collected from each plot at random, no cotton grown within six feet from all the boundary lines being included, in order to avoid the boundary effect. Each sample is a representative of the bulk sample grown under each of the farmyard manure series plots.

In all twenty-four samples, namely, six from 1931-32, six from 1933-34 and twelve from 1934-35 season were examined. In the first two seasons, samples from the original plots only were obtained and all of them were from the first picking. In the last season 1934-35, samples of the second picking and grown under both the original and duplicate plots were received.

Original plots . .	1	2 1/2 tons per acre to cotton
	2	2 1/2 tons per acre to *jowar*
	3	5 tons per acre to *jowar*
	4	10 tons per acre to *jowar*
	5	No manure.
	6	15 tons per acre to *jowar*
Duplicate plots . .	6	15 tons per acre to *jowar*
	5	No manure
	4	10 tons per acre to *jowar*
	3	5 tons per acre to *jowar*
	2	2 1/2 tons per acre to *jowar*
	1	2 1/2 tons per acre to cotton

FIG. 1. The layout of the farmyard manure experiment showing the position, size and treatments given to the plots, each of which measured one-tenth of an acre.

SAMPLING

Sampling, *i.e.* the obtaining of a small sample of cotton weighing only a few milligrammes, which can be considered truly representative of the bulk is rather a difficult problem. The importance has been discussed by Turner [1928]. Such methods are described by Calvert and Summers [1925], Koshal and Turner [1930], Clegg [1932] and very recently by Underwood [1935]. The method followed in the present study is approximately the same as suggested by these workers.

Each test sample was divided into two equal parts until there were thirty-two parts in all. From each of these groups, small bunches of fibres were drawn and cleaned one by one. These were drawn by hand and mixed together into a sliver. The hand sliver after drawing, mixing and doubling together was passed through a draw-box several times till a representative sliver having most of the fibres parallel and straight was obtained.

EXPERIMENTAL PROCEDURE

The following fibre-properties are dealt with in the present enquiry, *viz.* (1) mean fibre-length and fibre-length distribution ; (2) fibre-length irregularity per cent ; (3) mean fibre-weight per inch ; (4) mean fibre-strength (oz.) ; (5) fibre-maturity and (6) ginning percentage.

The experimental methods adopted for the determination of characters (1) to (4) have already been described in detail elsewhere by Nazir Ahmad [1933].

FIBRE-MATURITY

The method followed by Clegg [1932] was adopted in the present study with a slight modification, *viz.*, while Clegg takes fibres from different sections of a Baer Sorter pattern according to their length, here a bunch of fibres having nearly 300 fibres was drawn from a representative sliver. These were placed between microscopic slide and a cover glass and irrigated with eighteen per cent caustic soda solution. About 1,000 fibres were tested for each sample and the percentage of mature, half mature and dead hairs were calculated.

GINNING PERCENTAGE

The ginning percentage was determined by actually weighing the *kapas* before ginning and afterwards by weighing the lint and seed separately on a sensitive balance.

DISCUSSION OF RESULTS

The experimental results obtained by various fibre-properties are given in Tables I-VII.

MEAN FIBRE-LENGTH

The results obtained from these tests are given in Tables I and II and are graphically represented in Fig. 2.

TABLE I

Treatments	Seasons					
			1934-35			Mean for all seasons
	1931-32 (in.)	1933-34 (in.)	Original (in.)	Duplicate (in.)	Mean for 1934-35 (in.)	(in.)
No manure . . .	0·90	0·88	0·88	0·87	0·88	0·88
2 1/2 tons to *jowar* . .	0·88	0·89	0·92	0·89	0·90	0·89
5 tons to *jowar* . . .	0·91	0·89	0·91	0·93	0·92	0·91
10 tons to *jowar* . . .	0·89	0·85	0·88	0·87	0·87	0·87
15 tons to *jowar* . . .	0·92	0·87	0·91	0·85	0·88	0·89
2 1/2 tons to cotton . .	0·90	0·89	0·89	0·90	0·89	0·89

TABLE II

Mean fibre-length and fibre-length distribution of Jaywant cotton of the three seasons grown under various doses of farmyard manure

Sample No.	Fibre-length distribution percentages : Mean group lengths in eighths of an inch								Mean fibre-length (inch)		
	3	4	5	6	7	8	9	10	By Balls Sorter	Baer Sorter	Mean of Balls and Baer
1931-32 (original)											
s1	1·6	3·5	7·6	15·8	24·8	27·8	15·7	3·2	0·901	0·882	0·891
s2	1·9	3·3	7·1	15·3	31·1	32·3	7·5	1·5	0·881	0·868	0·874
s3	1·4	3·6	7·8	15·5	26·2	28·1	13·4	4·0	0·899	0·881	0·890
s4	1·4	2·8	6·8	15·0	26·4	29·1	15·4	3·1	0·908	0·895	0·901
s5	1·6	3·3	7·2	15·8	27·1	30·3	12·6	2·1	0·894	0·885	0·890
s6	1·2	2·1	5·3	13·5	27·3	31·9	16·6	2·1	0·921	0·911	0·916
1933-34 (original)											
s7	1·8	3·6	8·8	16·6	28·3	26·2	12·0	2·7	0·884	0·880	0·882
s8	1·6	3·0	8·1	15·6	28·1	29·4	12·6	1·6	0·890	0·871	0·880
s9	1·7	3·2	7·2	16·2	27·1	29·1	13·3	2·2	0·893	0·882	0·887
s10	1·5	3·6	8·0	17·0	28·5	27·3	12·4	1·7	0·886	0·875	0·880
s11	2·2	4·4	1·3	21·4	29·4	22·6	7·6	1·5	0·846	0·854	0·850
s12	1·5	3·8	8·0	19·0	29·6	25·3	11·0	1·8	0·875	0·859	0·867
1934-35 (original)											
s13	1·8	3·3	7·9	16·8	30·0	25·3	13·3	2·6	0·885	0·883	0·884
s14	1·2	2·6	6·2	12·0	26·5	30·8	17·1	3·6	0·923	0·898	0·910
s15	1·8	4·0	9·4	16·7	26·4	24·0	13·4	4·0	0·886	0·867	0·876
s16	1·8	4·0	7·7	14·2	24·9	26·6	15·8	5·0	0·905	0·903	0·904
s17	1·9	3·8	9·5	18·7	26·6	22·9	12·2	3·4	0·877	0·870	0·873
s18	1·5	2·7	7·0	14·6	26·5	27·1	16·4	4·2	0·912	0·908	0·910
1934-35 (duplicate)											
s19	1·3	3·3	9·2	20·4	30·9	23·4	9·2	2·3	0·868	0·863	0·865
s20	1·7	3·7	7·9	14·1	29·5	26·9	12·8	3·5	0·894	0·897	0·895
s21	1·6	3·1	6·9	14·6	27·4	28·2	14·3	3·9	0·905	0·893	0·899
s22	1·6	2·8	6·2	11·7	23·8	29·0	19·0	6·0	0·933	0·918	0·925
s23	2·1	4·5	10·2	17·7	25·7	24·3	12·1	3·4	0·874	0·852	0·863
s24	1·8	4·9	12·1	20·7	28·3	21·3	9·4	1·8	0·848	0·835	0·841

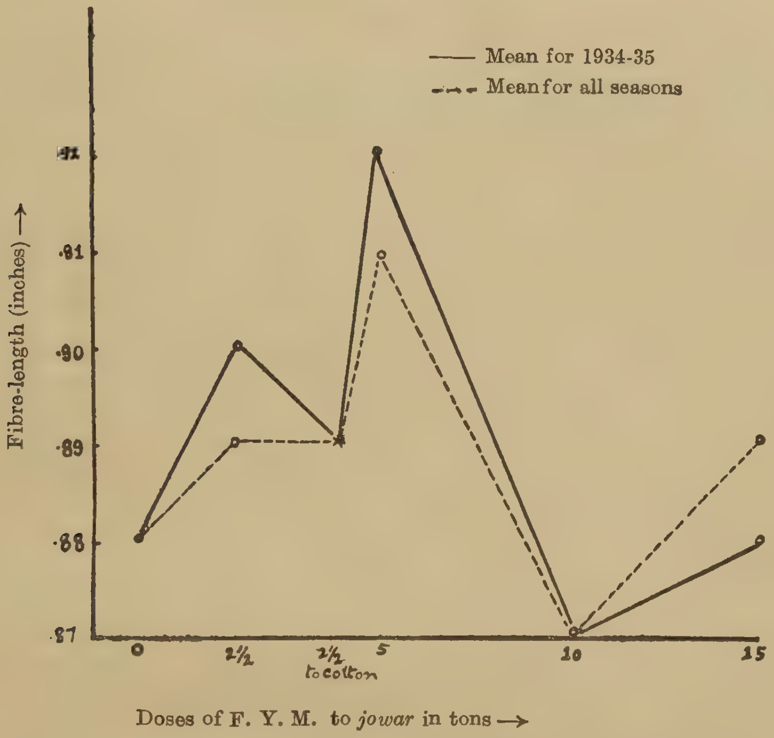


FIG. 2. Change of fibre-length with varying rates of farmyard manure (*represents 2½ tons F. Y. M. application to cotton).

It should be noted that the 1934-35 samples have this advantage over the others that in this season tests were made on duplicate samples. The results given in Table I for the 1934-35 samples show that the best conditions for fibre-length are obtained when five tons farmyard manure was applied to *jowar*, the smaller and heavier doses both brought about a certain amount of deterioration in fibre-length, the former presumably due to soil starvation and the latter to overfeeding. This conclusion is supported by the means for all the seasons in which the seasonal effect is eliminated.

The conclusion reached by Armstrong and Bennett [1933] and Reynolds and Killough [1933] that there is no significant correlation between the length of fibre and the rates of application of fertiliser, therefore, appears untenable.

TABLE III

Values of the fibre-length irregularity of Jaywant cotton of the three seasons grown under different doses of farmyard manure

Treatments	Seasons					
	1931-32	1933-34	1934-35			Mean for all seasons
			Original	Duplicate	Mean	
No manure . . .	16·9	15·9	12·3	11·8	12·0	14·9
2 1/2 tons to *jowar* . .	14·8	15·5	13·2	16·1	14·7	15·0
5 tons to *jowar* . . .	15·1	14·2	15·2	15·7	15·5	14·9
10 tons to *jowar* . .	15·8	15·9	13·8	15·2	14·5	15·4
15 tons to *jowar* . .	13·5	13·1	12·1	17·2	14·1	13·6
2 1/2 tons to cotton . .	15·9	15·5	16·6	14·5	15·5	15·6

The mean values for the two sets of samples of 1934-35, as given in Table III, show a tendency for the fibre-length irregularity to increase up to five tons farmyard manure to *jowar*, and to decrease afterwards. This tendency is also shown by the mean for all the seasons, though not by the individual seasons. This is in line with the observed increase in the mean fibre-length up to five tons farmyard manure to *jowar* and a decrease afterwards, as it has been found by Nazir Ahmad and Navkal [1933] that, as a rule, longer cottons possess higher fibre-length irregularity in cotton.

The curves, relating to the mean of the two sets of samples for the 1934-35 season and to the means for all the seasons are given in Fig. 3.

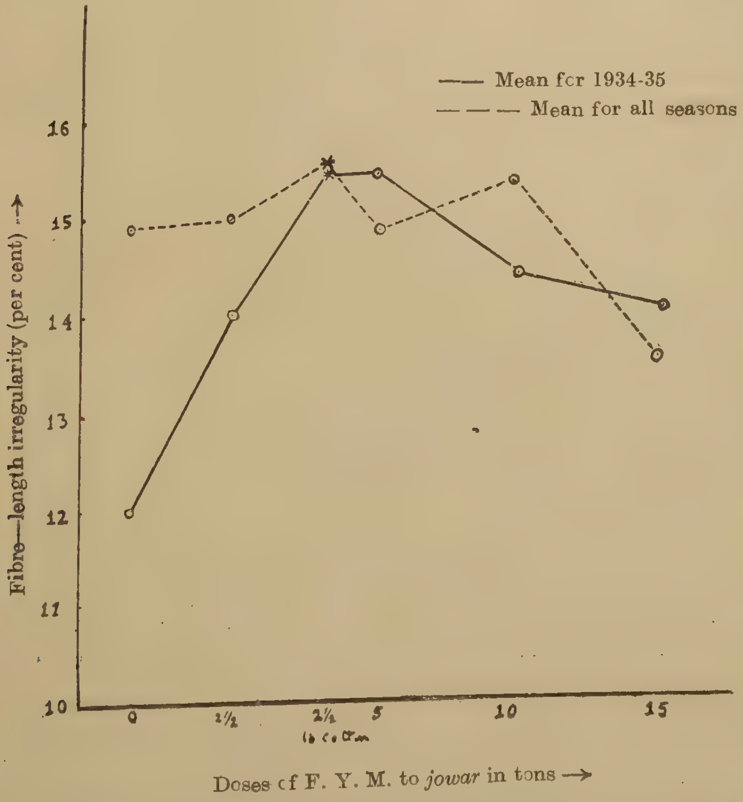


FIG. 3. Change of fibre-length irregularity with varying rates of farmyard manure (* represents 2½ tons F. Y. M. application to cotton).

MEAN FIBRE-WEIGHT

TABLE IV

Mean fibre-weight results of Jaywant cotton of the three seasons grown under various applications of farmyard manure

Treatments	Seasons					
	1931-32	1933-34	1934-35			Mean for all the seasons
			Original	Duplicate	Mean	
No manure	0·175	0·152	0·149	0·130	0·140	0·156
2 1/2 tons to *jowar*	0·158	0·149	0·134	0·154	0·144	0·150
5 tons to *jowar*	0·150	0·143	0·158	0·142	0·150	0·148
10 tons to *jowar*	0·151	0·137	0·116	0·120	0·118	0·135
15 tons to *jowar*	0·147	0·134	0·138	0·112	0·125	0·135
2 1/2 tons to cotton	0·169	0·142	0·126	0·148	0·137	0·149

From the data recorded in Table IV, it is clear that the fibre-weight per inch tends to decrease with the increasing rate of farmyard manure. In the year 1933-34, the decrease is quite steady, the percentage difference between the coarsest sample grown with no-manure and the finest grown under fifteen tons farmyard manure per acre being about twelve. The values for the samples of 1931-32 season have the similar trend with the exception of the sample grown with 2 1/2 tons to cotton. The percentage difference between the highest value obtained for the sample grown under no-manure and the lowest grown under fifteen tons farmyard manure is over seventeen. In the case of the samples of 1934-35 season grown under duplicate and original plots, there is again the same trend with some exceptions. The percentage variation between the highest and the lowest values of samples of 1934-35 season of both the original and duplicate plots are about thirty and thirty-one, respectively. These results agree with the conclusions reached by Pope [1935], who states that the percentage lint tends to decrease with the increasing rate of fertiliser, etc. It may be expected that the weight of individual fibres tends to decrease with the increasing rate of farmyard manure and hence conclusion may be drawn that the fibre-weight decreases with the increasing rate of farmyard manure applied. The difference between two successive values is also significant in many cases.

The mean values for the two sets of samples of 1934-35, and the means for all the seasons conclusively show that there is a tendency for the fibre-weight to decrease with the increasing rate of farmyard manure.

The effect of varying rates of farmyard manure on fibre-weight values for the different seasons is graphically represented in Fig. 4.

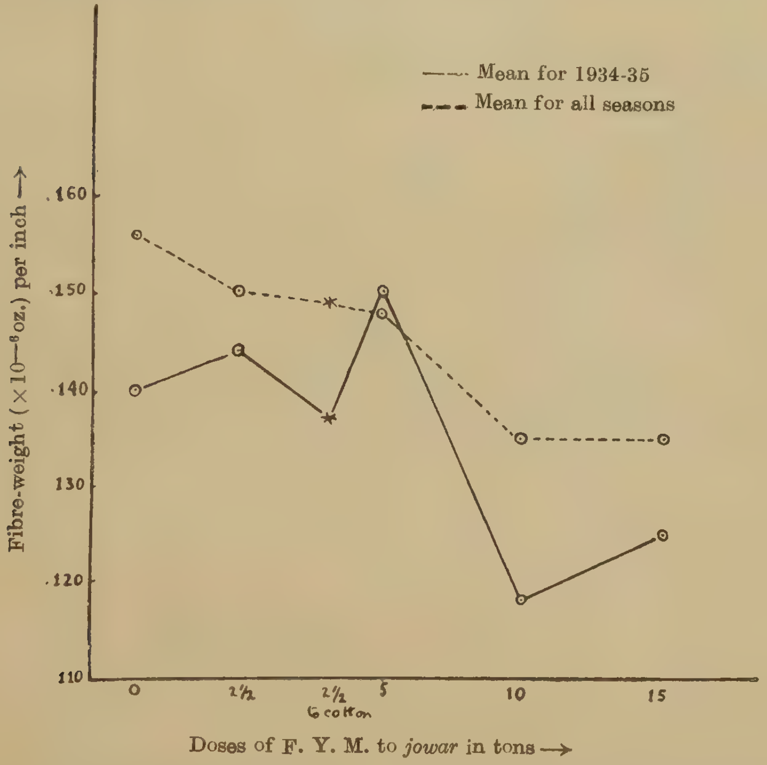


FIG. 4. Effect of varying rates of farmyard manure on fibre-weight (* represents application of 2½ tons of F. Y. M. to cotton).

TABLE V

Mean fibre-strength values of Jaywant cotton of different seasons grown under varying rates of farmyard manure

Treatments	Seasons					
	1931-32	1933-34	1934-35			Mean for all seasons
			Original	Duplicate	Mean	
No manure	0·144	0·125	0·149	0·130	0·140	0·136
2 1/2 tons to *jowar*	0·150	0·133	0·146	0·145	0·145	0·143
5 tons to *jowar*	0·144	0·131	0·139	0·134	0·136	0·137
10 tons to *jowar*	0·145	0·135	0·109	0·119	0·114	0·131
15 tons to *jowar*	0·163	0·134	0·132	0·114	0·123	0·140
2 1/2 tons to cotton	0·146	0·131	0·122	0·136	0·129	0·135

The results given in Table V indicate that the effect of the varying rates of farmyard manure application on this fibre property to be non-significant. Especially it is true in the case of the samples of 1931-32 season and also of 1933-34. But the results of both the original and duplicate plot samples of 1934-35 season show a decreasing tendency with the increasing rate of manure. In the case of the original plot samples the decrease is steady with the exception of two samples grown under 2 1/2 tons to cotton and ten tons to *jowar* respectively, these values being very low. As regards the samples from the duplicate plot of the same season the decrease is more steady with a single exception of the sample grown under no-manure. The percentage difference between the highest and the lowest fibre-strength values of the samples of both the original and duplicate plots of 1934-35 season are thirty and twenty-four respectively.

The mean values of the two sets of samples of 1934-35 and the means for all the seasons show a tendency for the fibre-strength to decrease with the increasing rate of farmyard manure. The conclusions arrived at with regard to samples of 1934-35 season and the means for all the seasons corroborate with the findings of Pope [1935] and other workers that the tensile strength indicates that a decreasing strength accompanies an increasing rate of application and that a decrease in strength is usually associated with more favourable growth conditions. These results are graphically represented in Fig. 5.

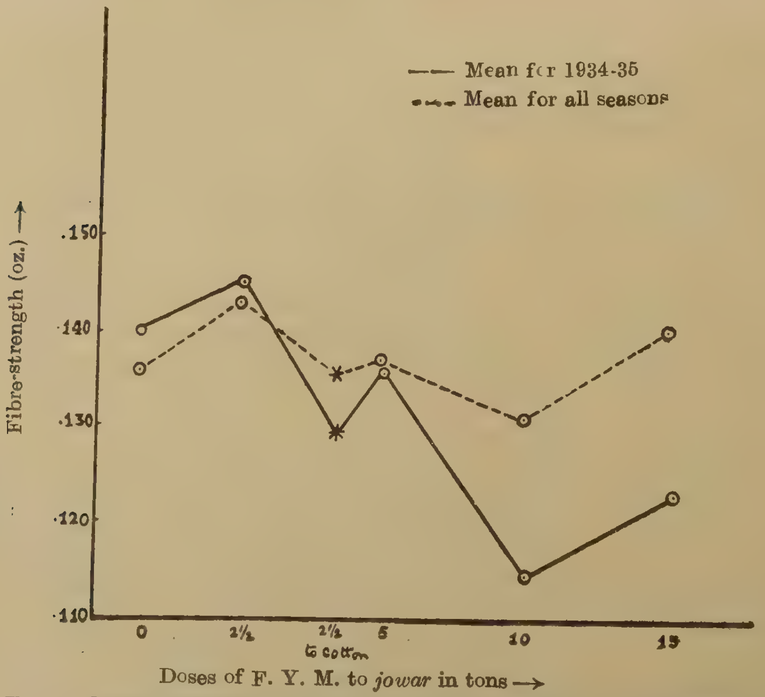


FIG. 5. Effect of varying rates of farmyard manure on fibre-strength (* represents application of 2½ tons F. Y. M. to cotton).

FIBRE MATURITY

TABLE VI

Fibre-maturity values of the different samples of Jaywant cotton grown under varying rates of farmyard manure

Treatments	Season 1934-35		
	Original	Duplicate	Mean
No manure	46—34	44—35	45—34
2 1/2 tons to *jowar*	47—36	56—31	51—33
5 tons to *jowar*	57—29	50—34	54—31
10 tons to *jowar*	41—43	44—34	43—38
15 tons to *jowar*	53—31	49—38	51—35
2 1/2 tons to cotton	41—42	55—32	48—37

From the above table, it is clear that the means of the original and duplicate plots show a tendency like the fibre-length values, for the number of mature hairs to increase up to five tons farmyard manure to *jowar* and to decrease thereafter.

These results are depicted in Fig. 6.

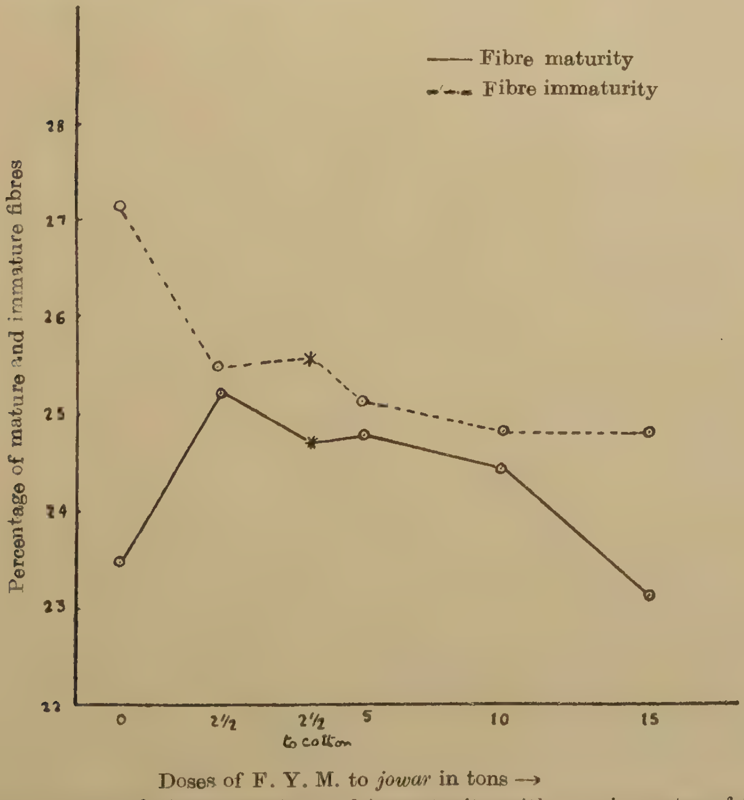


FIG. 6. Change of fibre-maturity and immaturity with varying rates of farmyard manure (* represents application of 2½ tons F. Y. M. to cotton).

GINNING PERCENTAGE

TABLE VII

Ginning percentage values of the different samples of the three seasons of Jaywant cotton grown under rates of farmyard manure varying from none to 15 tons per acre

Treatments	Seasons					
	1931-32	1933-34	1934-35			Mean for all seasons
			Original	Duplicate	Mean	
No manure	27·5	30·3	24·3	22·6	23·5	27·1
2 1/2 tons to *jowar*	26·1	25·0	26·6	23·9	25·2	25·4
5 tons to *jowar*	25·5	25·0	24·7	24·9	24·8	25·1
10 tons to *jowar*	25·1	25·0	24·8	24·1	24·4	24·8
15 tons to *jowar*	26·4	25·0	24·0	22·2	23·1	24·8
2 1/2 tons to cotton	26·7	25·2	24·0	25·4	24·7	25·5

The results recorded in the above table indicate that the ginning percentage of two samples of 1931-32 and 1933-34 seasons grown with no-manure are higher than those obtained for other samples grown under different doses of farmyard manure. But the two samples of 1934-35 grown with no-manure have given lower results which are quite contrary to the results of the previous seasons. While the values of the first two seasons go to show that the weight of lint is proportionately higher for the samples grown with no-manure than those grown with the varying rates of farmyard manure, the result of the third season does not quite agree with the conclusion.

The values obtained for the samples of all the seasons show that the ginning percentage decreases as the dose of manure increases, with of course a few exceptions. The means of the two sets of samples of 1934-35 and the mean for all seasons conclusively show that the higher rates of application contribute to decrease in ginning percentage. This decrease may be due to the fact that the seed grows well due to proper nourishment depending upon the rate of manure applied, thus accordingly increasing the seed weight and consequently lowering the ginning percentage. The rate of growth of seed seems to be more rapid than the growth rate of lint.

This somewhat agrees with the conclusion reached by Pope [1935] who states that the higher rates of application contribute to an increased seed weight and weight of seed cotton. Percentage lint tends to decrease with increasing rate of fertiliser, this being due to the relatively stable amount of lint per seed and a seed weight which increases with amount of fertiliser application.

Like other fibre properties mentioned in this paper, it is surprising to see that the values obtained for ginning percentage in the case of samples of the original and duplicate series of 1934-35 season do not agree with each other. The reason for obtaining results with wide difference for two similar samples grown under the same kind of soil, identical conditions and treatments cannot be explained. The original and duplicate plots are identical in each and every way. There is no doubt that there must be one or more factors which are responsible for these differences in the results of the same cotton grown under similar conditions. The large difference noted between the original and duplicate samples of the 1934-35 season may partly be due to soil variation.

The results of the ginning percentage are graphically represented in Fig. 7.

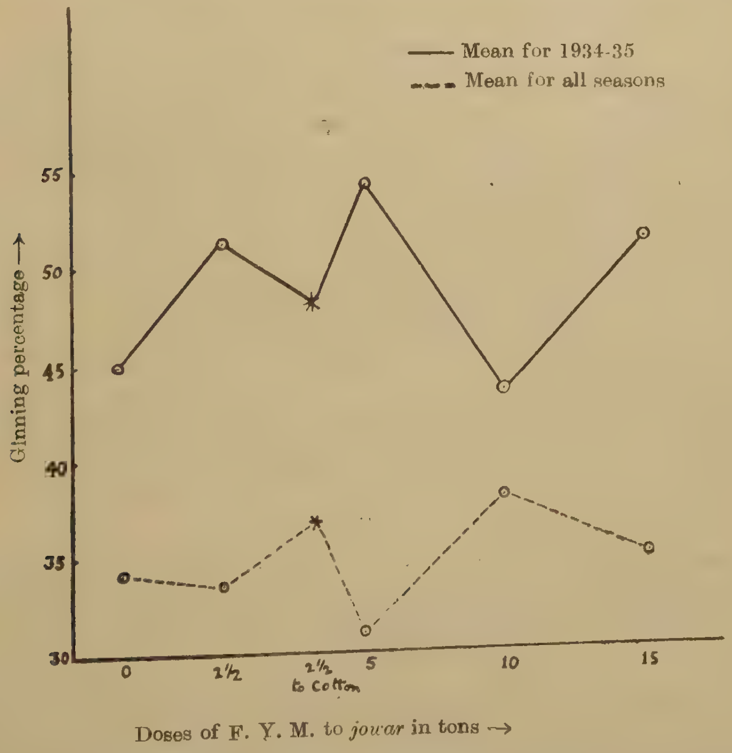


FIG. 7. Change of ginning outturn with varying rates of farmyard manure (* represents application of 2½ tons F. Y. M. to cotton).

CONCLUSIONS

The tests conducted during the three seasons to determine the effect of the application of the varying rates of farmyard manure on the fibre properties and the ginning outturn, indicate that there is practically not much variation in the fibre properties studied. It cannot be conclusively said that larger quantities of farmyard manure induce superior qualities. On the other hand comparatively poor results have been obtained for the samples grown with ten and fifteen tons. These results apply to Jaywant cotton, but it will probably hold good for other cottons also. As the quality of the cotton is not seriously affected by supplying more manure, it is evidently unnecessary to continue this type of work on the Experimental Farms, except perhaps to confirm these results and also if it is to be valuable from view of earliness, maturity, yield, stand, etc. It should however be pointed out that the optimum conditions with regard to the quality of cottons are produced with five tons farmyard manure to *jowar* (the previous crop to cotton), the lighter and heavier doses may bring about a certain amount of decline in quality. This amount is likely to vary with the local condition and past history of the soil.

ACKNOWLEDGMENTS

The writer would like to place on record his thanks to Rao Saheb G. L. Kottur, Cotton Breeder, S.M.C., Dharwar, for kindly permitting him to conduct this enquiry, to the Farm Superintendent for supplying the material and especially to Rao Bahadur S. S. Salimath, Deputy Director of Agriculture, S. D., Dharwar, for the keen interest and encouragement given in the work. Dr. Nazir Ahmad, Director, Technological Laboratory, very kindly supplied the Grader's report and helped with valuable suggestions and useful directions in preparing this paper for the press. His special thanks are due to Dr. B. B. Mundkur, Imperial Agricultural Research Institute, New Delhi, for critically going through the manuscript and for his suggestions.

The work was carried out under the financial assistance kindly rendered by the Indian Central Cotton Committee, Bombay.

SUMMARY

Jaywant cotton grown on the Dharwar Farm during the three seasons, 1931-32, 1933-34, and 1934-35, under varying rates of farmyard manure was studied for several fibre characters and ginning outturn and the following conclusions were arrived at.

The application of varying rates of farmyard manure show that the optimum conditions for the quality of cotton were obtained with five tons of farmyard manure to *jowar* which was the previous crop, while lighter and heavier doses brought about a decline in quality in one respect or the other.

The mean values of the two sets of samples of 1934-35 and the means for all the seasons show that the application of five tons farmyard manure to *jowar* yielded longer staple-length, increase in fibre-length irregularity and an increase in the number of mature hairs.

The mean fibre-weight values decreased with the increasing rate of farmyard manure, the difference between two successive values being also significant.

The values of the mean fibre-strength showed a decreasing tendency with the increasing amount of farmyard manure.

The ginning percentage showed a decreasing trend with the increasing rate of farmyard manure application.

REFERENCES

Armstrong, G. M. and Bennett, C. C. (1933). *J. Agric. Res.* **47**, 467-474.

Balls, W. L. (1915). "The Development and Properties of Raw Cotton". A & C Black Ltd., London.

Calvert, M. A. and Summers, F. (1925). *J. Text. Inst.* **16**, T238.

Clegg, G. G. (1932). *J. Text. Inst.* **23**, T 36-T54.

Gracie, D. S. and Fanny Khalil (1935). *Min. Agric. Egypt Tech. Sci. Serv. Bull.* **152**, 71.

Hawkins, R. S. and Serviss, G. H. (1930). *J. Agric. Res.* **40**, 1017-1029.

Koshal, R. S. and Turner, A. J. (1930). *Ind. Central Cotton Committee, Tech. Bull. (Series B)*, **6**.

Nayak, H. R. (1936). *Ind. J. Agric. Sci.* **6**, 52-62.

Nazir Ahmad (1933). *Ind. Central Cotton Com. Tech. Bull. (Series A)* **25**.

Nazir Ahmad and Navkal, H. (1933). *Ind. Central Cotton Com. Tech. Bull. (Ser. B)* **16**, 8.

Nazir Ahmad and Sen, D. L. (1934). "Effect of Manurial Treatment on the Yield, Fibre-properties and Spinning Value of a Cotton." Indian Science Congress, 1934.

Pope, O. A. (1935). *Bull.* **319**.

Reynolds, E. B. and Killough, D. T. (1933). *J. Amer. Soc. Agron.* **25**, 756-64.

Sturkie, D. C. (1934). *J. Amer. Soc. Agron.* **26**, 1-24.

Turner, A. J. (1928). *Ind. Central Cotton Comm. Tech. Bull. (Ser. B)* **1**.

Underwood, C. (1935). *J. Text. Inst.* **11**, T 309-T 335.

Youngblood, B. (1929). *Proc. Ann. Conv. Assoc., Southern Agricultural Workers*, 106-109.

THE INFLUENCE OF NON-NITRIFYING ORGANISMS ON NITRIFICATION

BY

S. V. DESAI, D.Sc., Ph.D., D.I.C.

Agricultural Bacteriologist, Agricultural Research Institute, Lyallpur

AND

FAZAL-UD-DIN, B.Sc.

Bacteriological Assistant

(Received for publication on 11th June 1937)

Nitrifying organisms are so extremely sensitive to soluble organic matter in pure culture that even minute traces inhibit their growth in culture media. Under natural conditions, nitrification occurs in the presence of large quantities of organic matter, as in manure pits, in sewage water, and in humus soils. The fact that organic matter is not toxic in soil and that such matter is toxic in pure culture signifies that some sort of modification in physiological activities of the nitrifiers is brought about by the presence of other bacterial flora. The decomposition of the organic materials through the action of other organisms is generally accepted as an explanation of the fact that soil organic matter is not toxic, although this fact has never been established by experimental evidence. The influence of non-nitrifying organisms on the process of nitrification has not yet been studied sufficiently to explain this anomalous behaviour. These experiments were started with a view to investigate this remarkable difference in the physiological activity of the nitrifying organisms.

At first, it was proposed to investigate the effect of different soluble organic substances on nitrification. For this purpose fifty c. c. of dilute Omeliansky solution (1 : 3) were taken in duplicate flasks with addition of different organic substances in requisite amounts and sterilized. Inoculations were made with soil suspension corresponding to 0·05 grm. of soil. The flasks were incubated at

30°C. and analysed after seven and fourteen days respectively. Mean values of the results are given in Table I.

TABLE I

Nitrification in Omeliansky solution in the presence of different organic substances (Amount in mg. of nitrous nitrogen per 100 c. c.)

Serial No.	Substance	Amount (equivalent to)	1st week	2nd week
1	Control	..	0·07	0·78
2	Soil extract	0·5 grm.	0·16	0·81
3	,, ,, ...	1·0 ,,	0·19	0·79
4	,, ,, ...	2·0 ,,	0·39	0·86
5	,, ,, ...	5·0 ,,	0·26	0·91
6	Manure extract . . .	0·5 ,,	0·10	0·84
7	Cane sugar	0·2 per cent	*Nil*	Trace
8	Glucose	,,	,,	0·5
9	Lactose	,,	,,	Trace
10	Mannite	,,	,,	0·06
11	Dextrine	,,	,,	0·50

In the first week, no nitrification was observed in the presence of carbohydrates. In the second week, nitrification started and the carbohydrates were found to be absent when tested for. Presumably nitrification started after the destruction of the various carbohydrates by the contaminating organisms. The addition of soluble organic matter in the form of soil extract and manure extract to Omeliansky solution did not show any inhibitory action on nitrification.

In order to determine the influence of non-nitrifying organisms on nitrification, a purified culture of *Nitrosomonas* obtained by repeated platings on silica plates and numerous transfers in Omeliansky solution was used as an inoculum. The culture was found to have one contaminant similar in appearance to *Nitrosomonas*, but which grew very slowly in bouillon. This contaminating organism had very similar characteristics to *Nitrosomonas* but it was unable to bring about nitrification and had practically no influence on the behaviour of *Nitrosomonas* in the presence of organic matter.

Fifty c. c. of the following three media were distributed in different flasks and sterilized

Medium No. 1

Horse-dung extract	1000 c. c.
Ammonium sulphate	0·5 grm.
Potassium phosphate	1·0 ,,
Calcium carbonate	10·0 ,,

Horse-dung extract was prepared by autoclaving 100 grms. of horse-dung with 1000 c.c. of water for half an hour, and filtering.

Medium No. 2

Omeliansky solution	250 c.c.
Distilled water	750 c.c.
Dextrose	2 grms.
Calcium carbonate	10 grms.

Medium No. 3

Ashby's mannite solution	100 c. c.
Ammonium sulphate	0·5 grm.
Calcium carbonate	10.0 ,,
Distilled water	900 c. c.

One flask of each medium was inoculated with soil and incubated for three days. The films appearing on the surface represented the micro-organisms that rapidly multiplied in the media, and these served as inocula of non-nitrifying flora. Six flasks of each medium were inoculated with one c.c. of the purified culture of *Nitrosomonas* out of which three flasks of each medium were further inoculated with a loopful of the scum from the corresponding flask inoculated with soil. Three more flasks of each medium were inoculated with a loopful of the scum only. All the flasks were incubated at 30°C., and analysed after a fortnight. The flasks inoculated with the scum in addition to the nitrifying culture, contained more nitrite than those which were not inoculated with the scum (Table II).

TABLE II

Influence of non-nitrifying organisms on nitrification
(Amount in mg. of nitrogen per 100 c. c. solution)

Serial No.	Medium	Inoculated with	
		Nitrifying bacteria	Nitrifying bacteria and non-nitrifying bacteria
1 2 3	Horse-dung extract medium	*Nil* ,, ,,	0·61 0·51 0·15
4 5 6	Mannite medium	Trace 0·65 0·97	3·56 3·40 3·56
7 8 9	Dextrose medium	0·39 0·45 0·50	3·24 2·92 2·59

The flasks inoculated with the scum only did not show any nitrification, thus eliminating the possibility of any change in the quality or quantity of the nitrifying flora by the addition of the scum. The results clearly show that non-nitrifying micro-organisms play an important role in the process of nitrification.

The examination of the inoculum that stimulated nitrification showed the presence of bacteria as well as protozoa ; the bacterial flora was first studied.

BACTERIA

The scum obtained from the horse-dung medium inoculated with soil was plated out on nutrient agar for isolation of pure cultures of different types of bacteria. Five different organisms were selected for studying their influence on nitrification.

Fifty c.c. of the horse-dung medium were taken in each of the twenty-one conical flasks of 300 c.c. capacity and sterilized. These were inoculated with one c.c. of the nitrifying culture. One c.c. of emulsified suspension of the non-nitrifying bacteria was inoculated in triplicate flasks. One c.c. of suspension containing all the five organisms was added to another series of flasks. Three flasks were kept as control. The results given in Table III show that different organisms differ in their power of destroying the toxicity and stimulating nitrification.

TABLE III

Nitrification in the presence of different bacteria

Serial No.	Bacteria	Nitrous nitrogen (mg.) per 100 c.c.
1 2 3	Control	*Nil*
4 5 6	No. 1	0·24 0·19 0·11
7 8 9	No. 2	0·06 0·10 ..
10 11 12	No. 3	0·56 0·91 0·42
13 14 15	No. 4	0·97 1·23 0·97

TABLE III—*contd.*

Serial No.	Bacteria	Nitrous nitrogen (mg.) per 100 c.c.
16	No. 5	0·32
17		0·50
18		0·49
19	Mixed culture . . .	6·80
20		3·89
21		3·89

The mixture was much more efficient than the individual organisms. In order to find out whether this property of stimulating nitrification was in any way related to ammonifying power of the organisms or to the amount of their growth in the medium, further experiment was carried out. It was found that the property of destroying the toxins was neither proportional to their ammonifying power nor to their growth.

The effect of varying inocula of non-nitrifying organisms on nitrification in horse-dung medium was studied next. It was found that nitrification was influenced by the number of non-nitrifying organisms introduced with the inoculum. The periodical determinations of nitrous nitrogen showed that nitrification was not uniform with varying inocula of the non-nitrifying flora, but it increased with the number of the organisms introduced with the inoculum (Table IV).

TABLE IV

The effect of varying inocula of non-nitrifying organisms on nitrification (Amount in mg. of nitrous nitrogen per 100 c. c.)

Serial No.	Inoculum in c. c.	3rd week	4th week
1	*Nil*	*Nil*	*Nil*
2			
3			
4	0·01	*Nil*	*Nil*
5			
6			
7	0·05	*Nil*	Trace
8			
9			
10	0·1	Trace	1·68
11			2·01
12			2·59
13	1·0	2·75	4·86
14		3·40	5·51
15		3·72	5·18

PROTOZOA

All the three media described above allowed quite a good growth of protozoa. These were distributed in fifty-c. c. portions in conical flasks and sterilized. One flask of each medium was inoculated with the soil and examined from day to day for growth of protozoa. When the growth appeared, the scum was thoroughly shaken up in water, filled in a capillary tube and examined under the microscope. The emulsion was suitably diluted so that it was possible to have one protozoan in a centimeter length of tubing. The diluted culture was filled in long capillaries, which were cut in pieces of one centimeter length. These were carefully examined under a microscope and divided into those with protozoa and those without them. The flasks of media were divided into two similar sets. One set of flasks was inoculated with capillaries containing protozoa and the other with those without protozoa. Each flask was inoculated with four capillaries, which were crushed to release the enclosed organisms. All the flasks were inoculated with one c. c. of *Nitrosomonas* culture. After two to three days incubation at 30°C. the scum was examined to determine the accuracy of the inoculations. The flasks supposed to be without protozoa were occasionally found to be contaminated with protozoa and *vice versa.* Such flasks were discarded. The remaining flasks were analysed after a fortnight.

The protozoa in the dextrose and mannite media were similar. In the horse-dung medium a small type of protozoan was present in overwhelming numbers, whereas the bacterial growth was poor.

The results in Table V show that protozoa have no effect on the process of nitrification. Nitrification in horse-dung medium was extremely variable. Because of a rich protozoan growth, the scum had to be enormously diluted inorder to arrive at the proper dilution. As the bacterial growth was poor, the uniform quality of the inoculum was affected by high dilution. Besides the small type of protozoan mentioned above, other types were also present in some of the flasks. Although the results are so variable yet they show that the influence of protozoa on nitrification is negligible.

TABLE V

The effect of protozoa on nitrification
(Amount in mg. of nitrous nitrogen per 100 c. c.)

Serial No.	Treatment	Medium No. 1	Medium No. 2
1	Without protozoa . .	0·08	2·59
2		0·26	2·90
3		2·66	3·40
4		1·23	2·11
5		0·16	2·11
6		0·65	2·75
7	With protozoa . . .	0·23	2·43
8		0·23	2·43
9		2·81	2·75
10		0·15	2·92
11		0·31	2·59
12		2·38	2·59

YEASTS

Fifty c. c. of dextrose medium were distributed in each of twelve flasks of 300 c. c. capacity and sterilized. All the flasks were inoculated with one c. c. of the nitrifying culture. Half the number were inoculated. in addition, with a loopful of a pure culture of yeast (*Saccharomyces cerevisiae*) from dextrose agar. The flasks were incubated at 30° C. for a fortnight and analysed. The results (Table VI) show that the yeast helps nitrification by destroying sugar.

TABLE VI

Effect of yeast on nitrification

Serial No.	Treatment	Nitrous nitrogen (mg.) per 100 c. c.
1	Without yeast	0·97
2		0·97
3		0·84
4		0·55
5		0·71
6	With yeast	2·15
7		1·16
8		2·07
9		1·94
10		1·29

Having thus established that the non-nitrifying flora was essential to bring about nitrification in the presence of soluble organic matter, it was considered necessary to elucidate further as to how the non-nitrifying flora helped the nitrifiers. In order to determine whether the non-nitrifiers simply removed the toxic organic matter or lived in symbiosis with the nitrifiers, the following experiment was laid out :—

Fifty c. c. of the horse-dung medium were distributed in forty-eight 300-c. c. conical flasks which were weighed and sterilized. Half the number of flasks were inoculated with emulsified cultures of non-nitrifying organisms prepared as follows. All the five organisms used in the previous experiments were transferred to nutrient agar slopes ; after the cultures had grown for four days, these were emulsified with five c. c. of sterilized water, and transferred to a sterilized flask containing twenty-five c. c. of water. The emulsion was thoroughly mixed. One c. c. from this emulsion was used as an inoculum of non-nitrifying flora. All the flasks both inoculated and uninoculated were put in an incubator at 30°C. After two months, half the number of inoculated flasks were sterilized. The loss of moisture was made up with sterile water in all the flasks. These were arranged in four sets and inoculated according to the following scheme.

Initial treatment	Treatment after two months	
Inoculated with non-nitrifying bacteria	1. Sterilized 2. Unsterilized	Inoculated with nitrifying bacteria
Uninoculated	3. No treatment 4. Inoculated with non-nitrifying bacteria	Inoculated with nitrifying bacteria

The type of non-nitrifying organisms and the method of inoculation were the same as used in the previous experiments.

The flasks were incubated at 30°C. and analysed every week. The flasks after each analysis were rejected. The results (Table VII) show that the presence of non-nitrifying organisms is essential for promoting nitrification in a solution containing soluble organic matter.

TABLE VII

Showing the mode of action of non-nitrifying organisms

Serial No.	Treatment	Nitrous nitrogen (mg.) per 100 c. c.			
		1st week	2nd week	3rd week	4th week
1	Non-nitrifying bacteria grown for two months, sterilized and inoculated with nitrifying culture.	*Nil*	*Nil*	*Nil*	*Nil*
2					
3					
4	Non-nitrifying bacteria grown for two months and then inoculated with nitrifying culture	0·65	2·14	2·85	4·21
5		0·72	2·72	2·98	4·21
6		1·30	2·79	2·85	4·22
7	Inoculated with nitrifying bacteria	*Nil*	*Nil*	*Nil*	*Nil*
8					
9					
10	Inoculated with non-nitrifying bacteria and nitrifying culture	*Nil*	*Nil*	Trace	0·36
11				0·32	0·64
12				Trace	0·38

They also show that if the non-nitrifiers are eliminated by sterilization even after allowing them to grow in the medium for two months, the medium still remains unsuitable for the growth and activity of the nitrifiers and it is essential that both nitrifiers and non-nitrifiers should co-exist in the medium for bringing about nitrification. Further, non-nitrifiers by their growth render the medium suitable for nitrifiers, as is shown by the initial lag in the nitrification in flasks inoculated with non-nitrifiers and nitrifiers simultaneously, and this lag is absent from the flasks in which the nitrifiers were introduced after the non-nitrifiers had been allowed to grow in them.

Thus there are two factors which stimulate nitrification. The first is the removal of the toxic organic substances from the medium and the second is the co-existence of the two types, non-nitrifiers and nitrifiers in symbiosis with each other. In the above experiment the advantage of co-existence to nitrifiers is brought about, and to find out whether the introduction of nitrifiers was beneficila to non-nitrifiers as should be, in case of true symbiosis, further experiments were laid out.

Fifty c. c. of the horse-dung medium were distributed in sixteen conical flasks and sterilized. These were divided into two lots. In one lot the flasks were inoculated with one c. c. of the emulsion of non-nitrifying bacteria as described before. All the flasks were kept in an incubator at 30°C. After a fortnight the second lot was also inoculated with an emulsion of non-nitrifying bacteria. Each lot was further divided into two equal parts. One part in each lot was inoculated with one c. c. of culture of nitrifying organisms and the second part with one c. c. of sterile Omeliansky solution to balance the nutrients carried with the inoculum. The number of non-nitrifying organisms was estimated by plating on nutrient agar. Triplicate plates were poured. The flasks once used were discarded. The results (Table VIII) show that nitrifying organisms stimulate the growth of other organisms.

TABLE VIII

The influence of nitrifiers on the growth of non-nitrifiers

(Millions per c. c.)

Incubation period (days)	Advance inoculation		Simultaneous inoculation	
	Without nitrifiers	With nitrifiers	Without nitrifiers	With nitrifiers
3	49	219	330	600
8	..	..	255	360
12	34	152	317	650
18	58	101	330	860

It has been established that nitrifiers and non-nitrifiers carry on their function in true symbiosis, each type helping the other.

These experiments further explain why nitrifying bacteria behave anomalously when being transferred in series in Omeliansky solution. The type of contaminant determining the nitrifying activity of the culture differs in different flasks when the inoculum is small and number of contaminants is limited.

SUMMARY

1. The influence of non-nitrifying organisms on nitrification was studied.

2. Three media containing glucose, mannite, and horse-dung extract besides other inorganic nutrients were chiefly used in this work.

3. Soluble carbohydrates checked nitrification in Omeliansky solution until they were decomposed by non-nitrifying organisms. Soluble organic constituents of soil and fermented manure did not, however, depress nitrification.

4. It was established that nitrifying organisms live in symbiosis with non-nitrifying organisms, and are thereby able to overcome the toxic effect of soluble organic matter.

5. A number of non-nitrifiers were isolated from soil. They were found to differ in their power of destroying the toxicity. Mixtures of different organisms had greater power in this respect than any single organism.

6. Nitrification increases with the increase in number of non-nitrifiers introduced with the inoculum.

7. Presence or absence of protozoa had no effect on nitrification.

THE BLACK BEETLE (*ALISSONOTUM IMPRESSICOLLE* ARR.), A PEST OF SUGARCANE IN MYITKYINA DISTRICT IN NORTHERN BURMA

BY

C. C. GHOSH, B.A., F.R.E.S.

Deputy Director of Sericulture, Berhampore, Bengal

(*Formerly Entomologist to the Government of Burma*)

(Received for publication on 3rd December 1935)

(With Plates LXXVII and LXXVIII and five text-figures)

INTRODUCTORY

THIS paper records observations and experiments in Myitkyina district extending over several years. The sugarcane at other places in Burma has not been observed to suffer appreciably from insect pests, except on rare occasions from termites when grown on sandy soils. But insect pests have proved very serious to sugarcane in this district. Sahmaw, the principal scene of the activities of the pests so far, is at a distance of 294 miles from Mandalay. The observations and experiments had necessarily to be carried out at some disadvantage with occasional visits to Sahmaw and the district and with the help of the Sahmaw Estate Managers, *viz.*, Mr. A. St. J. Macdonald at the beginning for a short time, Mr. J. A. Smith mainly and Mr. G. Temple during Mr. Smith's occasional absences. The writer has to acknowledge the greatest help from Mr. Smith for carrying out and recording observations and experiments.

The area under sugarcane in the district recorded in 1909-10 was only twenty-three acres. The establishment of an experimental farm at Hopin in 1914, trials here of sugarcane varieties from 1916 onwards, availability of sets from this farm and encouragement of jaggery making led to an expansion of cultivation. Still the area recorded under this crop in 1924 was only 179 acres.

The establishment of a white-sugar factory at Sahmaw about this time by the Burma Sugar Company led to a very rapid expansion of the area under this crop which by 1934-35 rose to 10,534 acres.

The cultivation has however been dogged by a serious insect pest, *viz.* a black beetle, known to science as *Alissonotum impressicolle* Arr. In fact this pest proved to be the principal deciding factor as to the profitableness or otherwise of cane cultivation on the Sahmaw estate. The factory was originally started with the hope of being able to obtain all its supplies from sugarcane grown on the estate. After about ten years during which the fight against this pest was the major item, the owners of the factory stopped all cultivation on the estate in 1935 and are taking their supplies from outside growers. The facility of transport by rail has enabled cultivation of sugarcane to be extended over the whole district to a length of about ninety miles from Myitkyina to Mohnyin.

The insect pest was at first present only at Sahmaw but has been observed to appear at other places and at Namti it has already proved serious in certain areas. If it proves as serious over the whole area as it did at Sahmaw (and there seems to be hardly anything to prevent the spread of its injurious activities) the problem will be a very difficult one.

LOCATION AND GENERAL CHARACTER OF THE SAHMAW ESTATE

The estate of the Burma Sugar Co. at Sahmaw is a level stretch of land situated in the Namyin valley between two hill ranges and measuring about three miles along the length of the valley and about five miles across. The place is characterised by deficient drainage over the major part. Part of it was a boggy swamp which was drained later on and brought under cultivation. Before cultivation was undertaken, practically the whole area was covered by a thick growth of elephant grasses, mostly *Saccharum arundinaceum* Retz. (*kaing* in Burmese) and also *Imperata arundinacea* Cyrill. (*thetke* in Burmese). The place thus resembled the Kamrup Farm in Assam in general characteristics. About 1915-16 an experiment was carried out for about two years on drainage and cultivation with not very encouraging results.

SOILS

In the Sahmaw estate several types of soil are present :—

(*1*) *Heavy clay soil in A and B and part of C divisions.*—It had mostly *kaing* grass on it and when *kaing* was cleared there was a profuse growth of *thetke* grass. A part of this soil with heavy texture was observed to be not so suitable for the breeding of the beetle. Still there was attack but not very serious. In fields 28 to 30 in section I of A division up to fifth ratoon was obtained while fields with medium and light soils were suffering heavily.

(*2*) *Light loamy soil in Gravel Ridge and part of C division.*—Its behaviour as regards *kaing* and *thetke* grasses was similar to that of the above. This soil was observed to be very suitable for the breeding of the beetle which was always bad here.

(*3*) *Red laterite soil in Gravel Ridge West and Nampaung area.*—In the whole estate only this place had scrub jungle with *kaing* grass and a few trees. The beetle bred profusely in this area.

(*4*) *Soil like peat soil in a large swamp which was brought under cultivation after very elaborate and efficient drainage.*—After cultivation, drainage and breaking down of organic matter, the upper six inches of it became a loose, easily inflammable mass. The lower part however remained like boggy clay and the place would shake when walked on. The swamp had the tallest thick *kaing* grass in the whole estate. At first there was no beetle in it but later on there was attack here also.

Outside Sahmaw also different types of soil are met with in the places where sugarcane is being grown.

The Pyindaw area commencing from the fringe of the Nampaung area is higher than the Sahmaw estate and of the nature of low *taungyas* or low undulating hills. The soil is similar to that of Nampaung (*3*) above, but unlike Nampaung the place is heavily wooded and without any *kaing* grass. Sugarcane is being grown here since 1931 and although it is within about three miles of infested areas no beetle has appeared here yet.

At Namti, a station about seventeen miles from Sahmaw the soil is rather light clay loam. The beetle has already proved to be as bad as at Sahmaw in some fields. Hopin, about twenty-three miles from Sahmaw, has a sandy loam soil and has not suffered. All other places have an alluvial soil which does not resist the beetle. In the neighbourhood of Myitkyina the beetle has proved serious at one place at Ywadaw.

In texture the soils in Gravel Ridge and parts of C division in Sahmaw estate resemble those of the outside stations where cane is being grown. It would appear that the beetle can breed in these stations.

The beetle has not been so far found to be injuriously active in highly acid soils in the district which are however liable to infestation by the sugarcane Woolly Aphis (*Oregma lanigera* Zehnt). In sugarcane-growing tracts at other places in the province there are soils which are neutral or even alkaline like highly infested Sahmaw soils. The pest is not present in these localities. But its behaviour can hardly be predicted if it finds access into those places.

The chemical analysis of soils infested and as yet uninfested hardly gives any definite clue to the secret of the activity of the pest. The only point which can at present be recorded with accuracy is the coincidence of presence of elephant grasses in both the infested places in Assam and Burma. At both these places the rich growth of the elephant grasses led to the assumption that sugarcane, a cousin of this grass, would grow. And in both places elephant grasses and the beetle have gone together. But it should also be recorded that the beetle has not remained confined to any particular area and has occurred on high well-drained lands without any elephant grass.

METEOROLOGICAL CONDITIONS

The rainfall at Sahmaw from 1926 to 1934 averaged 80·27 inches per year Fig. 1). At Hopin, twenty-three miles to the south, the average annual rainfall for ten years from 1915 to 1924 was 70·28 inches. At Myitkyina, forty-eight miles to the north of Sahmaw, the yearly average for ten years ending 1934 was 90·40 inches.

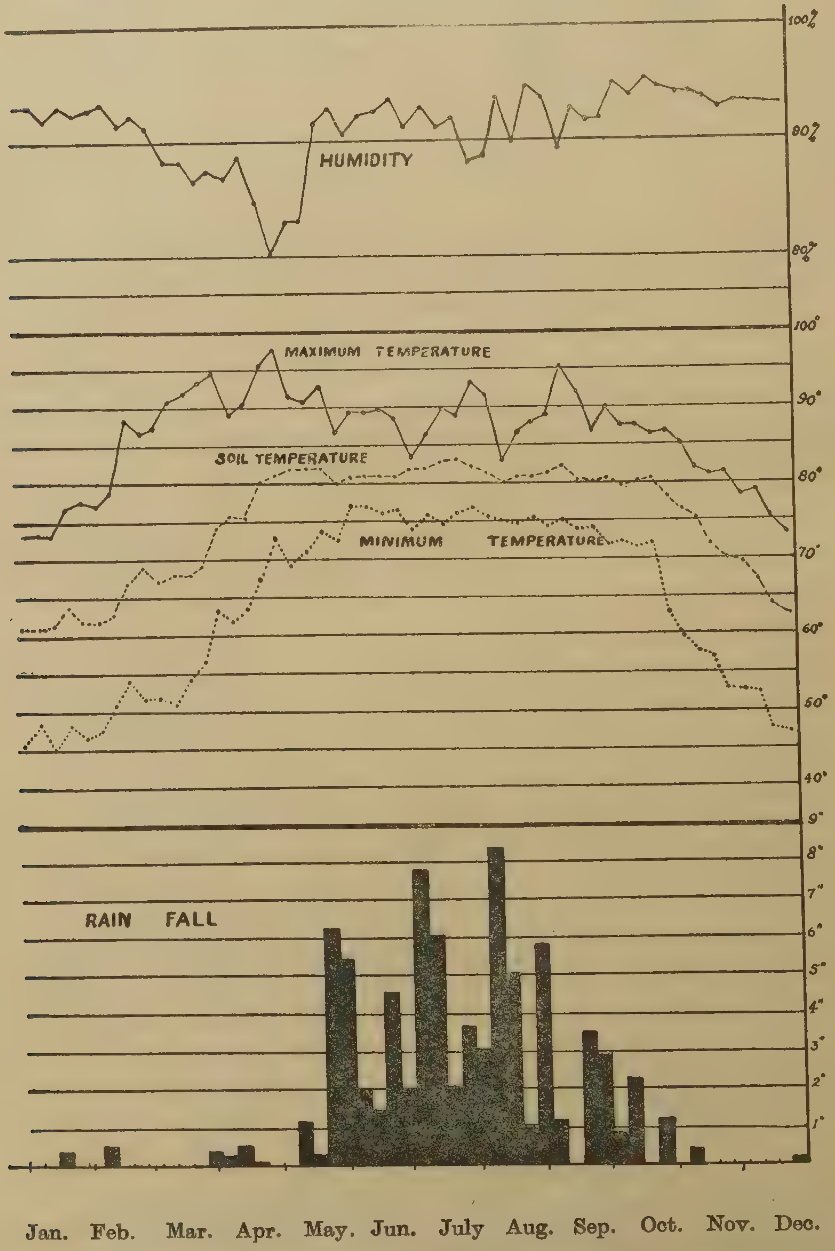


Fig. 1. Meteorological conditions at Sahmaw during 1933 (weekly averages)

The maximum and minimum temperatures, relative humidity, soil temperature and rainfall at Sahmaw for the year 1933 are shown in a graph prepared from the records of the Burma Sugar Co., on averages calculated for every week.

One important point to be noted in connection with the climate of the locality which has an important bearing on ripening of cane is the prevalence, from about the middle of November to the end of February, of fog in the whole of the cane-growing area every morning till about midday.

The beetle pest in the adult stage becomes active with the commencement of the rains and remains so till the early part of July, that is, well into the period of fairly heavy rainfall. During the period of heavy rain it remains inactive, resuming activity at the close of the rains. The grubs are active during the rainless period.

The other injurious pest, the sugarcane Woolly Aphis is active at the close of the rainy season and increases enormously in the early cold weather.

HISTORY OF THE PEST

The beetle first appeared in a pest form in April 1919 in the Sugarcane Experiment Farm at Kamrup in Assam [1918-20]. This farm, about 800 acres in area, was situated in the midst of an extensive piece of waste land running along the base of the Bhutan Hills and having on it a thick growth of tall elephant grasses, *Saccharum* spp. The farm had been started in 1915 and the beetle which appeared in injurious form four years later, was investigated in May 1919 at the farm and studied in the insectary at Pusa [Ghosh, 1921].

The pest appeared in seriously injurious numbers at Sahmaw in April 1927 and a thorough collection and destruction of the beetles was carried out. In 1928, 1929 and 1930 its activities did not call for very serious attention but collection and destruction of the beetles were carried out every year. From 1931 however the position became very serious necessitating closer study which revealed the highly injurious nature of its activities both in the larval and adult stages. It is the most injurious of all the pests on this crop so far observed and capable of doing enormous damage in a very short time.

OCCURRENCE OF THE PEST IN OTHER COUNTRIES

This beetle is known to occur as a pest of sugarcane in Formosa where it causes damage in unirrigated canes in the southern districts. In the irrigated northern districts where sugarcane is rotated with rice hardly any damage occurs. The following points from a communication of Mr. Shuzo Takano, Entomologist, Government Sugar Experimental Station, Tainan, Formosa to the writer are of interest in connection with the behaviour of the pest at Sahmaw.

In Formosa (*a*) ratooning of cane is very rare, (*b*) the beetle causes the most damage in May-June, (*c*) cane planted in January-April suffers severely, while that planted in July-October suffers little, most of the planting being therefore done in July-October, (*d*) the pest is bad in Southern Formosa where cane is not an irrigated crop, (*e*) in Northern Formosa cane is an irrigated crop and grown in rotation with rice and further the rainy season in this part from December to March kills off the majority of the grubs, thus preventing the pest from becoming serious, (*f*) the parasitic wasp, *Elis annulata,* the same as the one occurring at Sahmaw destroys only about 1·5 per cent of the grubs.

Contrasted with the above the conditions in Burma are continuous ratooning and almost total absence of rain in the period of larval activity from December to March.

LIFE-HISTORY AND HABITS (PLATES LXXVII AND LXXVIII)

Details of life-history as observed in Assam [Ghosh, 1921] have agreed with those noted in Burma with slight modifications as to periods of activity as shown in Fig. 2.

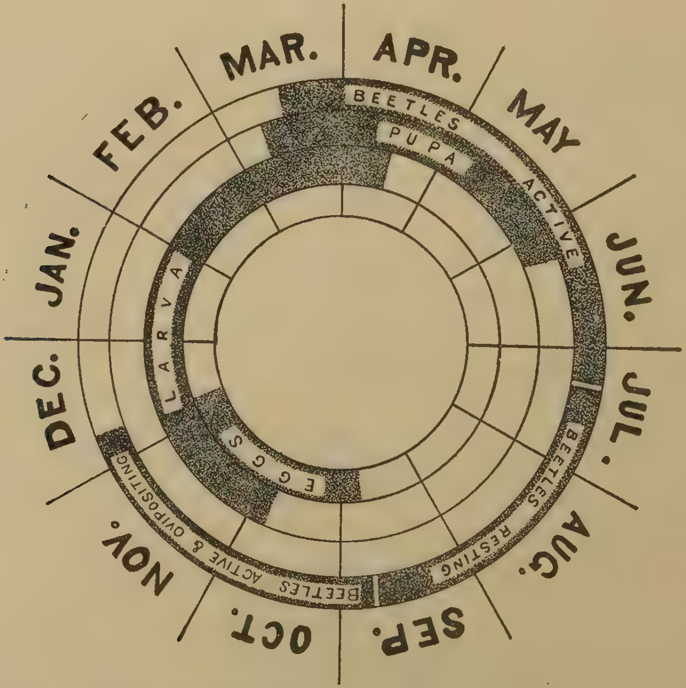
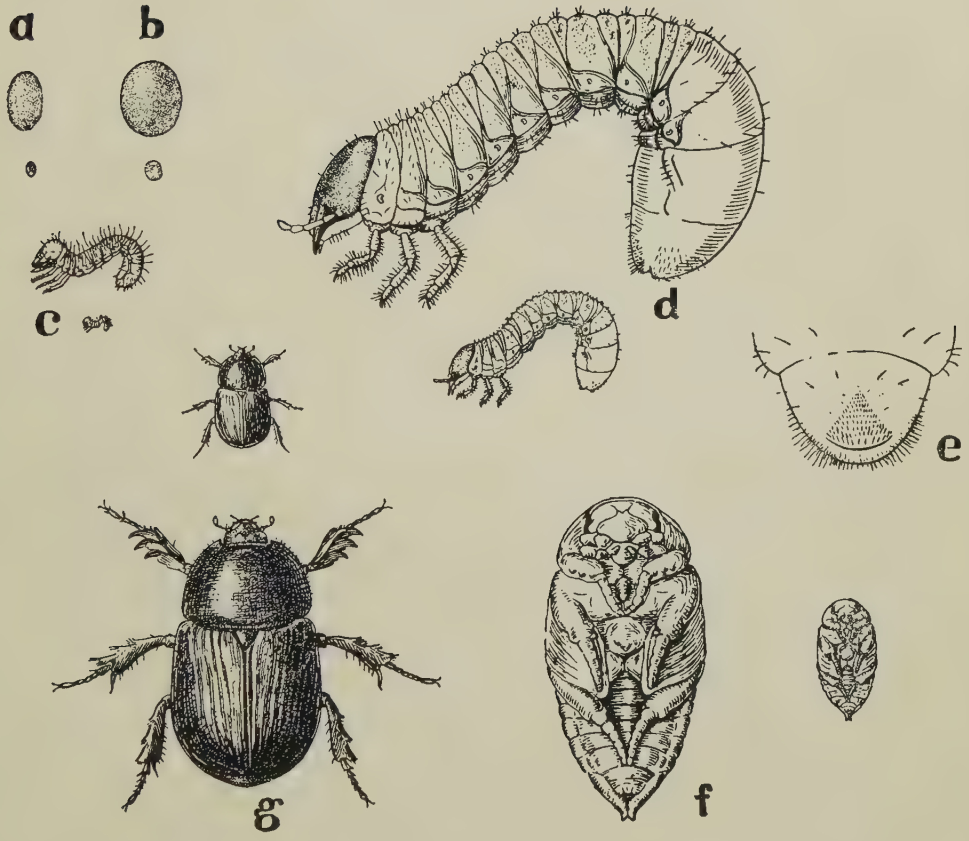


FIG 2. Annual seasonal life-history of *Alissonotum impressicolle.*

Eggs are laid in the soil from about the end of September till about the beginning of December and hatch in about two to two-and-a-half weeks. The grubs are capable of feeding and developing on humus but in sugarcane fields feed on roots, some becoming full grown and ready to pupate by about the beginning of March. The pupation period is lengthened, like the egg-laying period. Adult

PLATE LXXVII

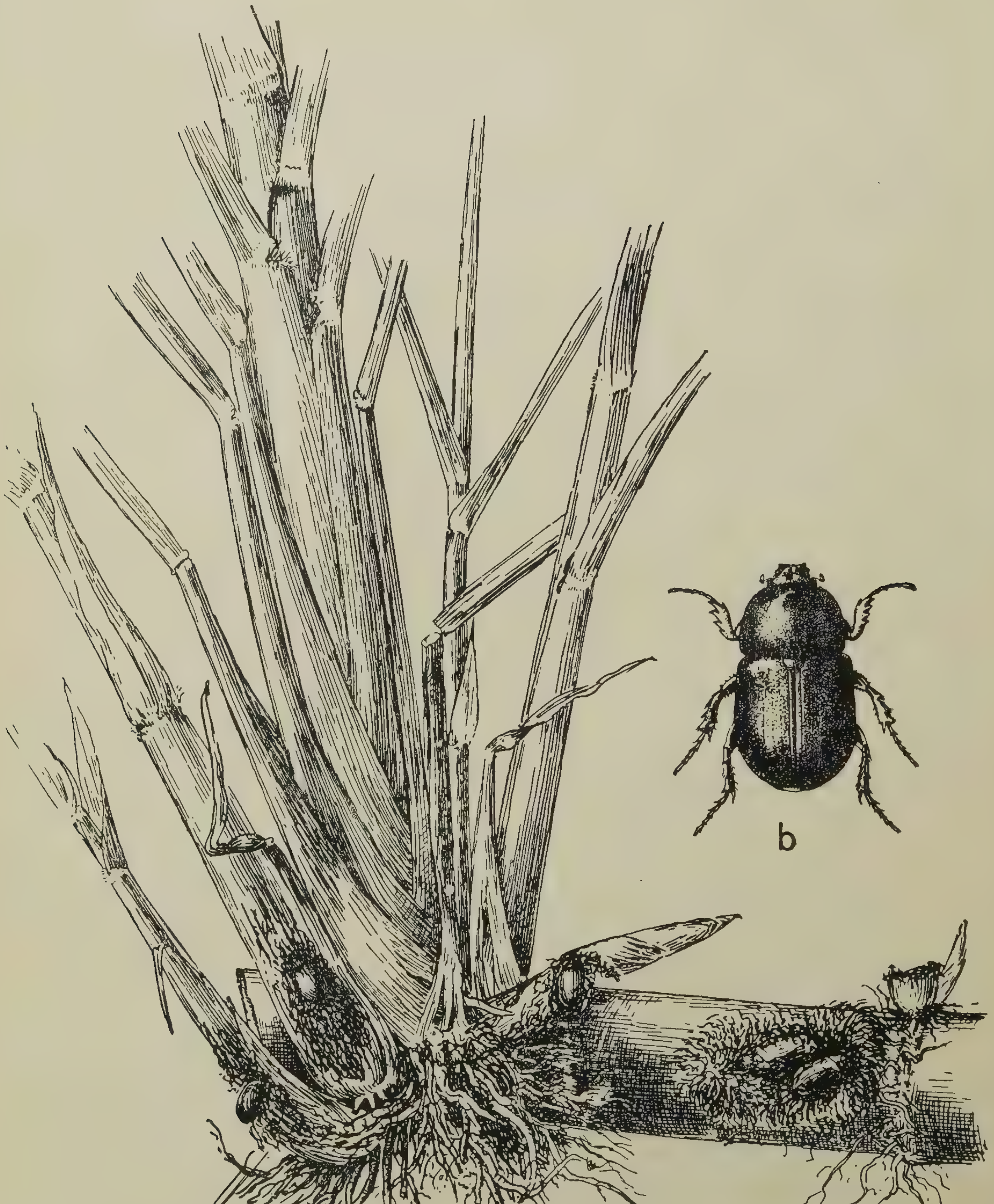


Alissonotum impressicolle (C. S. 1949).

a, Egg freshly laid (×4).
b, Developed egg before hatching (×4).
c, Newly hatched larva (×4).
d, Full-grown larva (×2½).
e, Spines on the ventral surface of posterior extremity of larva (×4).
f, Pupa (×2½).
g, Beetle (×2½).

(The smaller figures show the natural sizes.)

[Reproduced from the *Sci. Repts. of the Agric. Res. Inst., Pusa*, 1919-20.



Dynastine beetles damaging sugarcane at Kamrup.

a. A stool of sugarcane shoots with the sett, showing how the beetles bore into the sett and gnaw across shoots, grown as well as newly emerging.

b. A beetle (*Alissonotum impressicolle*).

[Reproduced from the *Sci. Repts. of the Agric. Res. Inst., Pusa*, 1919-20.

beetles begin to emerge by about the middle of March. They are active and cause damage by gnawing the bases of shoots or gnawing into sets in the absence of shoots, till about the end of June or beginning of July. Then they go down into the soil and rest, resuming activity again towards the end of September when eggs are deposited in the soil. They die off by December.

Normally the beetle lives and breeds among *kaing* and *thetke* grasses. It has never been found under *dub* (*Cynodon dactylon*) grass. Sugarcane roots and the cultivated conditions of the soil in sugarcane fields afford it highly favourable conditions for breeding and multiplying. The grubs live among and feed on cane roots and are therefore not affected by inter-culture between cane rows. When ready to pupate they have been observed to go down to a depth of up to eighteen inches and some apparently go down still deeper. On attainment of adult state the beetles come up and gnaw shoots. As very large numbers of grubs occur in a field many beetles are necessarily found together, giving an impression of swarming. This beetle has not been observed to fly although it walks freely over the fields at night. Mr. Smith, however, reported that about ten per cent of the beetles observed in evenings in April 1932 had their hind wings slightly protruded. This would indicate that either they were about to fly or had just alighted from flight. A strict watch over the active period failed to detect any swarming. It becomes a pest and causes serious damage only when conditions are favourable for its breeding in the field and such conditions are brought about when ratooning is continued long.

The activity of the beetles themselves has a very close connection with rainfall. April to June is the period when they are very active and feed voraciously. Still if there be rainless droughty periods at this time their activity and feeding decrease and are renewed with heavy showers of rain. The same holds good for the active period in autumn.

Although many beetles may be present in a field of grown-up cane their presence can hardly be detected until the cane is harvested and new shoots grow. It is the new shoots which are gnawed at the base. Shoots are even gnawed off before they appear above ground.

That the beetle migrates from field to field and from cultivated fields to surrounding areas, cultivated or uncultivated, has been very well observed. When a large grub-infested area, about forty-five acres, in Nampaung was ploughed up, prepared and left fallow for sometime before planting, apparently want of food led the adult beetles to migrate in large numbers. They crawled over the ground aimlessly and showed no inclination to fly. Many were intercepted by a railway line lying across their path and many fell into a straight-edged drain. Many were observed to have taken shelter in adjoining grasslands. In the same field after a heavy shower of rain in May following a prolonged droughty period, 181,000 beetles were picked up from the surface. The conditions in this case were rather abnormal and such large migration does not probably take place from unploughed fields or fields having a crop standing on it. Observation, however, at different hours at night has revealed beetles crawling aimlessly on the ground.

When a beetle crawling on the surface of the soil meets a shoot, it immediately burrows down at its base. In ploughed and cleared bare fields *Saccharum* grass stalks stuck into the ground in the path of beetles crawling on the surface immediately made them stop and burrow into the soil at the base of the stalks.

A strong light has rather a repellent effect on the beetles.

PROPORTION OF THE SEXES

During the egg-laying period in 1932 from the 10th September to 23rd November, 1,317 beetles were dissected for determination of sex and out of them 1,071 were females and 246 or 18·6 per cent males. The sexes are alike externally and dissection had to be resorted to in order to determine the sex. Later on a simple method was found out. If the abdomen be pressed between fingers applied to the two sides the internal terminal organs come out as a soft whitish fleshy mass through the anal end of the male. Nothing comes out in the case of the female. This method made determination of the sex in large numbers easy.

In a lot collected on 8th April 1933 there were 362 females and 290 males, that is, the sexes were about equal in number. But actual counts of different lots aggregating a total of 23,456 between 11th and 29th April showed only 1,338 or about 5·7 per cent to be males. Counts for the whole month showed 7·07 per cent to be males. In May 1933 out of a total collection of 403,453 only 4,865 or 10·7 per cent were males. In June 1933 in a lot of 59,975 only 9,938 or seventeen per cent were males. In the beginning of July in a lot of 489 only thirty-two or seven per cent were males. On 3rd October when the beetles resumed activity for egg-laying the first collection proved to have eighty per cent of the beetles as gravid females. The same proportion was observed to prevail throughout October and of the total collection of 49,664 during the month only 10,192 or twenty per cent were males. In November out of a lot of 596 only sixty-seven or twelve per cent were males. On 14th April 1934 in a small collection 113 were female and eighty male. Just at the commencement of activity in spring there were large numbers of males compared with females. But very soon females vastly outnumbered males.

OVIPOSITION

The beetles have been observed to lay eggs in the laboratory about October-November. In 1932 their behaviour in this respect was watched in the field. They were active from about 10th September to 23rd November. A number of them were dissected every few days during this period to find out whether eggs were present in the abdomen of the females. Between 10th September and 20th October mating couples were very commonly met with under the soil and during the whole period of activity females were found without eggs, with a few eggs and also with numerous eggs in their abdomen. The beetles are in the habit of laying only a few eggs at a time and often after intervals of days. Egg-laying was spread and continued over this whole period. The total number of eggs laid by a single female in nature has not been accurately determined and appears to be about 100.

In 1933 the beetles went down to rest before the middle of July and the earliest date of renewed activity for oviposition was observed to be 3rd October. In the interval however a few beetles could be met with occasionally. Activity continued throughout October during which 49,664 beetles, of which eighty per cent were females, were collected. Only 596 beetles were collected in November. Therefore the main ovipositing period was October. The position at the end of this month is evident from the statement below.

Date	No. of females dissected	No. without eggs	No. with eggs	No. with less than 10 eggs	No. with more than 10 eggs
25th Oct. 1933 .	735	..	735	380	350
26th Oct. 1933 .	335	40	295	55	240
27th Oct. 1933 .	361	31	330	..	..
28th Oct. 1933 .	220	19	201	..	..
30th Oct. 1933 .	109	29	80	..	..
31st Oct. 1933 .	525	96	429	138	185

Apparently there were many more beetles in the field ovipositing in the ground than came up and could be collected. Also the collection was only from fields with young cane where their activity and presence could be detected by the damaged shoots. In fields with tall grown canes their presence could not be detected and in all such fields egg-laying went on undisturbed.

DESTRUCTIVE CAPACITY OF THE PEST

In April 1927, when the pest first appeared at Sahmaw, there were 1,081 acres plant cane and 184·5 acres ratoon cane on the estate. Out of this about two acres plant cane and eleven acres ratoon cane were so badly affected that they had to be ploughed up. Of the rest about 18·5 acres ratoon suffered to the extent of about fifty per cent, about eighty acres plant cane ten per cent, and about 1,000 acres plant cane less than five per cent. About 200,000 beetles were collected and destroyed by the beginning of May from 203·5 acres at a cost of Rs. 5-10-6 per acre . The damage however continued and on 22nd May about twenty-three per cent of the stools were reported lost in about seven-and-a-half acres and about eleven to twelve per cent damage over the greater part of the area. Damage ceased in July. But a heavy shower of rain in October called forth the beetles again into activity and further damage was reported for a short period.

In 1928 attack was observed on 25th April but stopped by the beginning of May. The attack was all over the state but nowhere severe. Collection of the beetles was made but no record kept. Attack was slight in 1929 and also in 1930 when only about 15,000 beetles were collected. In 1931, however, the attack was very severe, about 200 acres of ratoon cane being wiped out in May and June when about 500,000 beetles were collected and destroyed. They were active again in October when about 4,000 beetles were collected. In the same year five acres were reported to have been wiped out at Ywadaw on the other side of the river at Myitkyina.

In March 1932 serious damage by grubs was discovered for the first time. A third ratoon field in Nampaung area had been observed since December to be going off-colour steadily and an examination in March revealed numerous grubs feeding on the roots. The damage by grubs was at first liable to be confused with the bad colour of the foliage of canes at Sahmaw due to the very dry weather at this time of the year. The attack was at its worst in March as the fully developed grubs were feeding heavily at this time before pupating. The first outward sign of the damage was the drying up of the leaves one after another until finally the centre shoot might die off and sometimes the plant toppled over having no longer any hold of the ground. Where grubs had been feeding on the roots the whole plant could be pulled out easily by hand and examination of the roots showed that the fibrous roots had been wholly gnawed off and the root stock bored and tunnelled, in most cases only a shell remaining. The surrounding soil was usually in a fine state of division and saw-dust like gnawings and pellets of excrement were mixed up with the soil.

Large areas of ratoon canes were affected in this manner and the standing cane dried up. In the case of one field with grown-up cane the harvesting of which had been delayed, the canes had to be abandoned having dried and become unfit for crushing. At this time examination of 37½ ft. to 100 ft. lengths of cane ridges in different fields revealed that about 40,000 to 144,000 grubs had occurred per acre, all feeding on cane roots and some were now pupating. Individual cane clumps had any number from about a dozen to six dozen grubs under each. This was the condition practically in all ratoon fields. Plant canes grown in already infested fields also suffered in the same way. Such condition continued to prevail in the state in spite of efforts at control.

Dates when activity of the beetle was first observed in successive years.

1927—25th April.
1928—13th April.
1931—14th April.
1932—26th April (Damage observed 18th March).
1933—24th March (Pupation observed to take place in the first week of the month).
1934—7th April.

These dates are connected with the first heavy shower of rain after about the middle of March. Even during the period of activity in summer and autumn heavy showers of rain are followed by extensive activity and consequent damage. This is due to the beetles being forced to come up by the soaking of rain water in the soil. Such activity could be brought about artificially in the laboratory by adding water to the earth in the troughs in which the beetles were kept.

Sugarcanes tried

The varieties of sugarcane tried and grown at Sahmaw are D 74, E K 28, B H 10-12, Striped Mauritius, Gilman (a sport of Striped Mauritius), P O J 2727 and 2878, F 74, S Mau, Pyinmana Red and Yellow, and Co 210, 213, 214 and 281. None of them showed perceptible immunity against the pest. But, generally speaking, the thin Co 213 was certainly more resistant than thicker varieties. This cane also grows, tillers and ratoons well with the minimum of cultivation and care and for these reasons was more suitable for the new Burman, Shan and Kachin cultivators and was encouraged till 1934. It spread everywhere. Up to the third ratoon of this cane is commonly taken and under favourable conditions fourth and fifth ratoons are grown. This long ratooning has helped the pest. The company has now definitely decided against this cane and are encouraging Striped Mauritius, Gilman and P O J 2714 and 2878. These ‘ noble ’ canes require better cultivation and care and cannot be carried beyond the first ratoon. This change may probably cause a little decrease in the area under cane for a time but is likely to improve the condition as regards the beetle pest as will be explained later on in the paper.

NATURAL ENEMIES OF THE BEETLE

The natural enemies of the beetle pest so far observed include birds, insects and two fungi.

Birds

The following four birds have been observed to prey upon the beetles, *viz.* Crows, Mynahs (*Acridotheres tristis*), Pied Harriers (*Circus melanoleucus*) and Wagtails (*Motacilia alba*). For the identification of the last two birds the writer is indebted to Mr. J. K. Stanford.

When beetles began to crawl on the ground in April-May 1932 crows were observed picking them up. One crow had seven beetles in its crop. Crows however were never observed to be present in large numbers or to scratch the earth for beetles. They are not therefore very keen on beetles but eat them casually when available. Crows and Mynahs followed the plough and picked up grubs exposed by the turning of the soil.

The Pied Harrier has been observed to dig in the earth for the beetle but the bird never occurs in large numbers.

The following observation on the 25th March 1933 indicates the habits of the Wagtail. At one end of an open verandah of a bungalow at Sahmaw there were side by side on a low wooden bench (1) *Alissonotum impressicolle* pupae in earth in an open collecting box, a few pupae exposed on the surface of the earth, (2) beetles in a bucket about half filled with earth, some of them moving on the surface of the earth, (3) grubs in a wide-mouthed open tin filled with earth, a few lying on the surface of the earth in a comatose state due to a drowning experiment and (4) twenty live beetles floating on the surface of water in a round open coffee tin about half filled with water. The writer was working at a table at a distance of about twelve feet Within one hour about midday one Wagtail picked off seven of the floating beetles, five beetles from the bucket and six of the exposed grubs but paid no attention to the prominent brown pupae. The grubs and beetles were picked up with the beak and swallowed.

The birds attack the grubs and beetles only when the latter are found exposed on the surface of the earth. The control exercised by the birds is very little.

Insect enemies

Insect enemies observed include an Asilid fly, *Philodecus femoralis* Ric., a Scoliid wasp, *Elis annulata*, a Tachinid fly, Carabid beetles and grubs and ants.

The Asilid fly.—The maggot of the Asilid fly preys upon the grub of the beetle. When an infested ratoon field was ploughed up at the end of March the cylindrical long white legless maggots of the fly lay exposed on the surface. Some could be observed actually feeding on the beetle grubs. The maggots however were very sluggish and apparently seemed to be unable to burrow down into the earth again. Therefore steps were taken to cover them with earth in ploughed-up fields and thus save them from being picked up by birds.

A number of maggots taken to Mandalay for observation pupated between 27th March and 7th April, adult flies emerging after fourteen to nineteen days from the date of pupation. The flies were liberated in large muslin cages and provided with water and live house flies on which they preyed and also with beetle grubs. They however laid no eggs and did not live for more than ten days.

These Asilid maggots were observed to be the best of all the natural enemies but as far as could be made out they were not responsible for the destruction of more than about two to three per cent of the grubs.

The Scoliid wasp, Elis annulata.—The wasp is of burrowing habit. One was found at a depth of about fifteen inches among the grub-infested roots of a sugarcane clump in an infested field. Another was found at a depth of about eight inches in similar situation and two were turned up by a plough working about seven inches deep in a grub-infested ratoon field. The wasp is very elusive and is hardly met with but has been collected in March, April, May and August at

Sahmaw. When kept in jars with earth and beetle grubs the wasps have been observed to pass most of the time under the earth only coming occasionally to the surface.

That the wasp grubs were preying upon the beetle grubs was evident from cocoons partly enclosed in the skin of the destroyed grubs. Ten cocoons were collected between 24th and 27th March, eight female wasps and one male wasp emerging from them between 16th and 22nd April. There has been no opportunity for getting the wasp to breed. From general observation the wasp did not seem to be responsible for destruction of more than about one to two per cent of the beetle grubs.

The Tachinid fly (unidentified).—About 1,000 grubs were received at Mandalay from Sahmaw on the 1st March 1932. From one grub ten fly maggots were obtained. The flies were reared and given a chance of oviposition on grubs but without success.

Carabid beetles and grubs.—Several Carabid beetles have been observed to be present in the sugarcane fields at Sahmaw. One Carabid beetle (*Scarites* sp.) and one Carabid grub have been definitely observed to prey upon *Alissonotum* grubs, but their number was not large.

Ants have been observed to attack beetles crawling on the ground but only occasionally.

Fungal enemies

The principal one is *Meterhizium anisopliae*. Out of about 1,000 *Alissonotum* grubs received from Sahmaw at Mandalay in March 1932 nine developed the fungus which had killed them. This is about the proportion observed in the fields at Sahmaw. The Mycologist, Burma prepared a culture which when tried in the laboratory did not justify any trial in the fields. The other fungus present is a species of *Cordyceps* (?) but distinctly of less efficacy than the above. The combined effect of the work of all enemies was not an appreciable check on the pest.

CONTROL

It has to be remembered that the pest is a soil insect working under the soil. As long as food is available it can live and multiply without coming to the surface. The adult beetles gnaw the bases of shoots below the soil surface and can be got at only by digging the earth. They mate and lay eggs in the soil and the grubs feed on roots and pupate deeper down. Enemies such as birds have therefore hardly any chance. The other natural enemies also do not exercise appreciable influence. When circumstances are favourable as when the cane is kept in the field for ratooning for two, three or four years, there is nothing to prevent the pest from breeding and multiplying undisturbed and hidden under the earth. The appropriate texture of soil which has been observed to have an inhibitory or deterrent effect is absent practically everywhere and in fact the soil practically at all places is suitable for breeding. Therefore, control can be effected either by not giving the pest a chance for breeding by restricting ratooning, or by finding some practicable method of killing it by means of collection or by application of insecticides. The adult beetles are active and move about at night and have not been observed to fly. From the number observed to breed and the number observed to move about it appears certain that only a small proportion of the beetles present in a field come to the surface under ordinary conditions. A large number of beetles was observed to migrate from an infested area which was wholly cleared of all plant growth and left fallow. As far as possible all beetles which could be got at were collected. Later on when cane was planted in the same area after a few months, damage to new shoots clearly indicated the presence of many beetles in it. Collection cannot be expected to get at all the beetles present in a field. Below are given short descriptions of the trials carried out for facilitating collection and also with insecticides.

Traps

Traps prepared by cutting kerosene oil tins lengthwise into two so as to have oblong vessels and also by cutting them similarly along opposite corners so as to have boat-shaped vessels, were placed on the ground and also sunk into the ground with the edges flush with the surface. They were used in areas where the beetles were active in October and baited with the following.

(1) Molasses with acetic ether just sufficient to impart a smell to the molasses.
(2) Fermented molasses.
(3) Acetic ether diluted in water
(4) Lactic acid diluted in water } about 1 in 5 of water.
(5) Formic acid diluted in water
(6) Amyl alcohol diluted in water
(7) Japanese Beetle bait reported to be successful in America and prepared as follows :—

Rice bran	1½ cups.
Molasses	2½ table spoonfuls.
Water	1 table spoonful.
Glycerine	1½ table spoonfuls.
Geruniol	4 table spoonfuls.
Oil pimento containing 90 per cent Eugenol (in place of Eugenol)	¼ tea spoonful.

None was at all effective.

In May 1932, when beetles crawled about on the ground, a galvanised sheet gutter 16 ft. long and 6 in. deep sunk level with the surface of the ground caught an average of thirty beetles daily and smaller ones about 6 ft. long sixteen daily. Kerosene oil tins cut diagonally and similarly sunk in the ground caught about five every night. Geranium and eucalyptus oils added to some tins did not show any advantage over untreated tins.

Split canes as baits

Beetles placed in earth in large glass troughs in the laboratory came up readily and fed on split pieces of cane placed on the surface of the earth with the cut surface downwards. The beetles clustered on the cut faces. In order to see if beetles could be enticed away in the field from new shoots by such split canes placed by the side of cane rows, between 8th and 18th April 1932, fresh split cane pieces numbering 792 were used in different fields at Sahmaw but only 128 beetles could be got. In addition to the above, thirty pieces of split canes and thrity pieces of split thick *Saccharum* grass stem were smeared with geranium oil but without any effect whatever. Split canes were also tried in June 1933 between cane rows where *Alissonotum* beetles and *Anomala bilobata* grubs were occurring in large numbers but without any effect. In a road drain by the side of a ploughed-up Nampaung field left fallow and from which the beetles were migrating at night, split canes gave some good results. One piece of soft juicy cane eighteen inches long had 120, 130 and sixty-eight beetles under it on three successive mornings. The conditions in this case were exceptional. The beetles always showed a preference for cane pieces to *Saccharum* grass stems used with cane pieces.

Insecticides

Insecticides in the form of fumigants of the soil so as to kill the grubs and beetles present in the soil among cane roots could be expected to be of use. The following trials indicate how far insecticides and fumigants are of use.

Cyanogas (calcium cyanide) A dust

A test carried out to determine its effect when applied to the soil at the base of cane stools, showed that applied at the rate of one ounce per running foot it had no injurious effect on the cane but at the rate of three ounces the leaves became bronzed and some shoots died.

Tests carried out with grubs and beetles in confined situations in the soil showed that cyanogas was fairly effective against both, provided it had a chance of acting on them. It caused about fifty-six to eighty-nine per cent mortality in such trials. Practical results in fields however were not encouraging. Applied at the rate of 80 lbs., 470 lbs., 560 lbs. and 700 lbs. per acre in furrows two to three inches deep on both sides of cane ridges and about six inches from the canes, the dust was observed to cause only 1·3 and eight per cent mortality in the second and fourth cases and none in the others. The fumes did not travel much and killed only those which happened to be near the dust when applied.

Water in which the dust was shaken up at the rate of ten and fifteen grammes to a gallon and which was poured at bases of infested clumps killed all grubs and beetles which it reached as it soaked down. Application in this form was not easy. Cyanogas therefore could not be used with success against the pest.

Carbon bisulphide

The use of carbon bisulphide is limited to light and loose soils and dry conditions, a combination of circumstances not always available. In compact clay soils the fumes do not travel and just below a cane ridge where it was injected with Vermorel Excelsior Soil Injector in April neither the grubs and beetles nor a termites' nest which happened to be present were affected.

When tried on grubs confined in boxes or in confined situations in soils it caused 90 to 100 per cent mortality. Applied at the rate of 70 lbs. and 120 lbs. per acre in three-inch-deep furrows made in cane ridges it caused only twenty-five and eight per cent mortality respectively in March.

In a ploughed-up loosened field it caused in March about seventy per cent mortality among grubs when injected with the Excelsior injector at the rate of five grammes one foot apart or about 200 lbs. per acre. Another field showed only forty-two per cent and a third field which had been swampy before only twenty per cent mortality in April with 168 lbs. per acre.

Anomala bilobata grubs which lived mostly within about six inches of the surface showed about fifty per cent mortality in September with 112 lbs. per acre.

The use of carbon bisulphide has therefore not been found to be economical and practicable.

Seekay

This soil fumigant was quoted at £72-10-0 per ton c. i. f. Rangoon which would amount to about Rs. 1,000 per ton at Sahmaw. The cost of treatment was calculated by Mr. Smith to be about Rs. 70 to Rs. 80 per acre, a prohibitive item. A small preliminary trial gave an impression that it might prove good as a repellent.

A preliminary trial with *Holotrichia* grubs kept in earth in a large glass trough in August showed that in such confined situations the stuff when fresh had some effect on grubs, twenty grubs treated dying within three days. Grubs introduced into the same earth treated eleven days ago were affected very slowly, some living for three weeks. A field trial at Mandalay against the same grubs under field conditions at 150 lbs. per acre gave absolutely negative results.

At Sahmaw a thorough trial was given against *Anomala bilobata* grubs which were occurring in enormous numbers and almost all of them within about six inches of the surface soil at the rate of 56 lbs., 112 lbs. and 168 lbs. per acre with absolutely negative results. At the last rate the cost of treatment would amount to about Rs. 75 per acre.

Potassium cyanide (ninety-eight per cent)

March 24th, 1932 (morning).—A cane ridge 37½ ft. long in a grub-infested 3rd ratoon field was treated as follows. A hollow bamboo tube with a stick inserted in it to serve as piston was thrust into the earth at the middle of the ridge, the piston pulled out, a lump of the poison dropped inside the tube, the piston re-inserted, the tube pulled out and the earth pressed down. About two oz. of poison was used. On 26th afternoon the ridge was dug to a depth of 7 in. The smell of the poison was perceptible in the earth. There were found six dead and seventy-nine live grubs, sixty-six live pupae and eighteen live beetles.

Paradichlorobenzene

(*1*) *Test of behaviour of paradichlorobenzene in soil (2nd November 1931)*.—Two crystals weighing 0·921 and 1·352 grammes were buried in a field. On 12th November the crystals were observed to have wholly disintegrated leaving a very strong smell which was perceptible even on 20th November at the place. Between 2nd and 12th there was 1·87 inches rainfall. Two crystals weighing 0·868 and 0·823 grammes similarly placed under soil in a field were reduced to 0·556 and 0·493 grammes respectively after five days without any rainfall.

(*2*) *October 28th, 1931* (5 P.M.).—Fifty beetles were buried in earth in a compound with two lumps of paradichlorobenzene placed about half an inch below the beetles. On the next morning at 8 A.M. there was a strong smell pervading the whole surroundings of the beetles of which one was dead, sixteen living and unaffected and thirty-three had escaped, their exit holes being observable.

(*3*) A number of bettles were placed in a large glass trough filled with earth and split canes in which crystals were embedded, placed on the surface of the earth. The strong smell did not deter the beetles which came up and simply covered the cane pieces on which they fed.

(*4*) *March 24th, 1932* (*morning*).—One and a half ounce crystals were placed under earth in the middle of a cane ridge in grub-infested third ratoon field in the same way as potassium cyanide crystals. On 26th morning the ridge was dug to a depth of about 7 in. and there were found sixty-nine grubs, forty-five pupae and twenty-four beetles all alive. There was a strong smell of the stuff in the soil.

Paradichlorobenzene and carbon disulphide

(*1*) *March 24th, 1932 (morning)*.—Paradichlorobenzene 3½ ounces was dissolved in eight ounces of carbon disulphide which was poured in two furrows 2 in. to 3 in. deep on both sides of a cane ridge 37½ ft. long in a grub-infested third ratoon field and the furrows immediately covered up with earth. On 26th morning the entire ridge was dug to a depth of fifteen inches and there were found fifteen dead and sixty-eight live grubs, twelve dead and eighty-one live pupae, twelve dead, two dying and thirty-eight live beetles. Mortality was eighteen per cent.

Little's Soil Fumigant

This proprietary stuff, similar to Vaporite but cheaper, used at the rate of three cwts. per acre in grub-infested ratoon fields at the time of ploughing up and clearing such fields was observed to bring about a mortality of about sixty per cent when examined four days after treatment. The treatment cost Rs. 25 per acre in thirty acres treated.

R. V. 4

This stuff said to be essentially sulphuretted hydrogen in the form of a coarse powder supplied by a Continental firm gave negative results against *Alissonotum*.

Effect of submergence

Twenty grubs were kept under water for twenty-six hours and when taken out three were dead, one was pupating and the rest were slightly bloated and stiff. In the course of about an hour the living ones revived and two bored down into the earth. Fifty-five per cent of them survived the submergence and the rest died.

Only one out of twenty pupae kept under water for four hours succumbed to the treatment. Submergence for twenty-six hours killed six out of eleven pupae.

The beetles are hardly affected by submergence as they quickly come up to the surface and float. The water may have a somewhat stunning effect causing some to float motionless with the legs fully stretched out. But beetles floating for ninety-six hours under observation did not seem to suffer from any ill effect.

On 24th March 1932 a portion of a grub-infested third ratoon field about 43 ft. × 30 ft. was flooded and water allowed to stand about two to three inches above the surface. It took several hours to let in the water and hold it by mounds raised all round. Flooding took place by about 12-30 P.M. By 1-0 P.M. sixty-two beetles and by 2-0 P.M. eighteen more came up to the surface of the water. Out of these eighty, five were still yellow in colour indicating that they had just attained adult state. On 25th March at 8 A.M. the water was let out. On 27th March evening one row of cane was dug out to a depth of fifteen inches and there were found seven dead and twenty-three live grubs, forty live pupae and two dead and five live beetles. The beetles had apparently attained adult state after the flooding.

Flooding forced out the adult beetles but could be of hardly much practical use as the beetles attained adult state in batches over a period of about two and a half months.

Along with the eighty *Allissonotum* beetles the following were flooded out, *viz. Heteronychus sublaevis* one, *Anomala* sp. two, *Balboceras* sp. one, Carabid beetles thirty-seven (two species), *Trochorrhopalus sacchari* one, Chrysomelid beetles thirty-four, Tenebrionid beetles ten and Pentatomid bug one.

Collection

16 [illegible]

Collection of beetles from infested fields is rather a laborious work as each beetle has to be dug out from a depth of about two to three inches of soil. In the Sahmaw estate according to intensity of attack one man could collect from a few to about 350 during the day. On some days during the active season as many as 150 coolies were engaged in collection and as the activity was spread over a long period the cane fields had to be gone over several times. The following is a statement of collections in 1932 and 1933.

Place	1932			1933		
	Area in acres	No. collected		Area in acres	No. collected	
		Summer	Autumn		Summer	Autumn
A-Division . .	428	423,627	118,044	..	..	..
Nampaung . .	105	487,319		264	172,427	38,179
Gravel Ridge . .	42	141,208		11	5,473	..
Gravel Ridge West .	204	92,662		243	355,355	7,562
C-Division . .	523	554,955		226	515,381	5,316
Collected from all places in March.	..	52,600		..	..	..
	1,302	1,752,381	118,044	744	1,048,636	51,057

See figures below under the section on general effect of clearing (pp. 926—928).

The above figures should be taken to indicate the activity of the beetles, intensity of attack, amount of damage and effort put forward to collect them. They represent only a part of the number present in the fields as will be evident from the enormous number of grubs present in infested fields. Collection cannot be expected to get all of them. Besides no collection was made from fields having grown-up cane as the beetles could not be located there.

In this connection the results of control by collection of a similar beetle pest, *Phytalus smithi*, in Mauritius may be compared. There the beetles are paid for and therefore presumably more efficient collecting was carried out. While in 1911 about twenty-seven million insects were destroyed the captures in 1930 amounted to 265 million beetles and 137 million larvae. Collection did not prevent increase of the pest but apparently prevented it from inflicting serious injury to cane cultivation which was reported not to suffer much in tonnage.

Collection and destruction are followed as a routine in the case of allied pests in all countries. The thorough collection carried out at Sahmaw in the very first year of serious attack in 1927 prevented the pest from attaining serious proportions during the successive three years. Ratooning, standing grown-up cane in the field, overlapping crops and other circumstances stood in the way of collection being effective and thorough. Still there is no doubt that collection exercised a good deal of control. Collection was looked after by the Company in the estate and in order that steps for collection might be taken when the pest appeared in outside stations a Sugarcane Pest Board for the district was formed. The scheme was to use the powers of the Company with respect to the granting of advance or the acceptance or refusal of cane, in order to insist upon cultivators taking necessary steps to control the pest, the steps being (1) collection of beetles and grubs from infested fields and (2) if necessary restriction of ratooning, clearing of cane fields and rotation or fallow.

A Pest Board was formed for the Nama area for protecting the interests of the local sugarcane growers and the local Sugarcane Growers' Association agreed to advance money for expenses to be incurred in collection of beetles and grubs at an interest of two per cent per mensem, the advance to be recouped from payments made by the Company to the growers concerned. This Board carried out collection once.

The scheme has not worked very satisfactorily at Namti.

Rotary plough

For clearing infested fields a rotary plough was imported from Australia, costing £100. It is made to be attached to a Fordson Tractor. It was found very useful in breaking up cane ridges and shaking out old cane roots. The earth and grubs are thrown out and strike against the metal covering, the grubs being thus injured. Two passages of the plough brought about about forty per cent mortality. Many grubs which escaped the blades lay on the top of the soil and were picked up by birds, mostly Mynahs and also Crows. Worked on cane ridges only, the ploughing cost Rs. 3 per acre. The plough works down to about four to six inches but can be made to go down about nine inches.

GENERAL EFFECT OF CLEARING, USE OF SOIL FUMIGANTS IN AND FALLOWING INFESTED FIELDS

Division C, Section II, Fields 7 to 21, situated side by side in Sahmaw estate were all along under *kaing* grass till 1929 when the *kaing* was ploughed up and the land left fallow allowing *kaing* to grow again. The fields were prepared and planted with cane in February 1930. Field 7 measured 8·25 acres and 17 and 21 measured 3½ and 3 acres respectively. The rest were equal in area being five acres each. In 1931 yield from them was about twelve tons cane per acre. The first ratoon yielded in January and February 1932 eighteen to twenty tons cane per acre. In March-April of the same year four fields, *viz.* 9, 12, 13 and 14 were wiped out by *Alissonotum* grubs. The third ratoon was harvested from the other fields in January 1933. In February 1933 all these fields were cleared with all reasonable care and planted. The rotary plough was worked twice over the cane ridges to a depth of about eight inches giving about forty per cent kill with two ploughings. Portions were treated with carbon disulphide injected by the Vermorel Excelsior Injector at the rate of five grammes every foot, killing about seventy per cent of the grubs in the treated portions. Some portions were treated with Little's Soil Fumigant at three cwts. per acre showing about sixty per cent mortality four days after treatment. Mynahs and Crows picked up grubs behind the plough. While ridging for planting four men behind the plough could collect only about fifty grubs in a day. Hardly better care could be taken in clearing infested fields. Germination was quite satisfactory. The shoots began to be attacked on 24th March.

A careful record was kept of the collections, number of men engaged and cost incurred. Taking all the fields together the average number of beetles collected from each acre during the year was 3,537 at an average cost of Rs. 14-12-0. The average cost of collecting 100 beetles was Re. 0-6-7. The average collection per acre in March-April was 1,110·6 by 6·5 men at a cost of Rs. 2-7-2, in May 800 by 8·1 men for Rs. 3-0-8, in June 1,445·5 by 15·7 men for Rs. 5-12-2, in July 475 by 2·7 men for Re. 1-0-2, in October 63·3 by 5·8 men for Rs. 2-1-0 and in November 5·7 by 1·1 men for Re. 0-6-11. Collections were made at the base of damaged shoots. There were certainly many beetles left in the fields.

Treatment with soil fumigants hardly made any difference in the number of beetles in treated and untreated fields. Different varieties of cane also made no difference. But fields 9, 12, 13 and 14 which had been left untouched after the ratoons in them had been wiped out in March-April the previous year and thus remained fallow for about a year, had much less beetles than even the contiguous fields on both sides of them in March, April and May. But by June this difference disappeared and this was apparently due to migrations from neighbouring fields.

That fallowing has a beneficial effect was indicated by the behaviour of two other fields described below. Fallowing must be for not less than a year.

An infested third-ratoon field (Gravel Ridge, Section 5, Field 2) eleven acres in area was observed to have grub, pupa and beetle population of about 67,000 per acre in March 1932. For the purpose of clearing it the field was disc-harrowed twice to a depth of about eight inches and cultivated once by steam tackle to a depth of about fifteen inches. Collecting was carried out simultaneously with each operation and a total of 10,230 was collected and destroyed, about one per cent being beetles, twenty per cent pupae and the rest grubs. The field was then left fallow till October 1932 when Co cane was planted in it. The cane grew without visible damage. Beetles collected from this find in spring up to the end of April 1933 numbered 1,154, in May 875 and in June 3,444.

One field of forty-five acres in third ratoon (Nampaung Section 5, Field 3) was cleared with a potato-digging machine and also steam tackle in March 1932 and left fallow for a few months and planted in June-July-August. Beetles were picked in October with some damage. Beetles picked in spring up to end of April 1933 numbered 24,265, in May 37,259, in June 28,355, in July 1,463 and in October 8,597. In this case fallowing was not sufficiently long with consequent damage.

CONCLUSION

The black Dynastine beetle, *Alissonotum impressicolle* Arr., is a serious pest of sugarcane in Myitkyina district in Upper Burma, both in the adult and larval stages, the larvae being more serious than the adult to ratoons and capable of killing off entire fields by gnawing away roots in the course of a few months from December to February. The adults gnaw the bases of cane shoots principally in April to June and to a smaller extent in October, the gnawed shoots being killed off entirely and not throwing tillers. Almost the whole of the damage caused to a field is by grubs and beetles bred in it. Beetles migrate from field to field principally by crawling over the ground at night.

The pest first appeared in Sahmaw estate of the Burma Sugarcane Company about 1927 and has made sugarcane cultivation unprofitable there, the cultivation of cane being given up in 1935. It has now appeared in all the sugarcane-growing tracts in the whole district and has proved as serious as at Sahmaw in some fields.

The pest is a soil insect living and working underground. The natural enemies present destroy less than about five per cent. Collection and destruction of beetles and grubs by digging them out at the base of damaged shoots and by ploughing up infested fields, carried out as thoroughly as practicable in the estate for two years did not wholly prevent increase of the pest or damage to cane. All sorts of measures which suggested themselves for control and eradication of the pest were tried in the estate. They proved costly and not practicable in the case of common cultivators and none was quite effective. The only method which has given a fairly satisfactory result is restriction of ratooning and fallowing. Therefore the method at present recommended is to take a plant and a ratoon crop and then to plough up and leave the field fallow for at least a year or grow a non-cereal crop on it. If cane is grown as an annual crop and rotated with non-cereal crops the beetle can be wholly overlooked. Ratoons are, however, profitable and if the activities of the beetle can be controlled, up to the third ratoon can be taken. As plenty of land is available in most places the method now recommended can be followed for some years. But it is certain that the pest will go on increasing and spreading as long as sugarcane is cultivated. In course of time the pest will probably be found everywhere and it will be impossible to escape damage by it and ratooning may have to be stopped wholly. Therefore, there is scope for introducing and trying parasites and predators from other places.

In biological control with the help of introduced parasites or predators, success has so far been achieved only in the case of introduced pests and not in that of indigenous ones like the one under discussion. The two notable cases of success in the case of allied pests are control of *Anomala orientalis* grub in Hawaii with an introduced Scoliid parasite, *Scolia manilae* Ashm. from the Philippines and that of the May Beetle in Puerto Rico by the giant toad, *Bufo marinus*, with regard to which the following note (*Nature*, Vol. XLIV, December 8, 1934, p. 877) explains the position.

> " From two of this species (*Bufo marinus*) brought to Puerto Rico from Barbados and Jamaica, millions of descendants have sprung and the food of this host has consisted largely of May beetle, the grubs of which known as white grubs devour roots of the cane. The planters formerly picked the grubs with hand. The introduction of the toad has reduced the May beetles to scarcity and the Puerto Rico sugar crop has been freed from its worst enemy."

The toad has been introduced into Mauritius, Hawaii and the Philippines and its introduction into Queensland is contemplated. In none of these places it has been sufficiently long to prove its usefulness. In Hawaii it has recently been reported to have multiplied amazingly.

In the case of the allied pest, *Phytalus smithi* in Mauritius, an introduced pest, parasites introduced have not so far been quite successful. But attempts at finding successful parasites and predators are continued. Similarly parasites introduced against the Japanese Beetle in the U. S. A. have not proved successful yet.

In the case of the beetle pest in Myitkyina the giant toad, *Bufo marinus*, should be given a trial first of all. It is likely to be more successful than parasites. Both Sir Guy Marshall and Dr. W. R. Thomson of the Imperial Institute of Entomology expressed doubts about the success of parasites in the present case and at their suggestion the Queensland authorities were consulted but they had nothing to offer.

There is no question about the pest being a potential menace to all sugarcane-growing areas in the province and there is necessity of an intensive study of it and its natural enemies on the spot and also scope for trying parasites and predators found useful on allied pests in other countries. A scheme for this purpose has been adopted by the Imperial Council of Agricultural Research but effect has so far not been given to it for want of funds.

What is required is prevention or minimisation of attack. Adoption of control measures after attack is practically impossible in the hands of the small village cultivators.

Other Dynastid pests

Several other Dynastid beetles are found working with and causing damage in the same way and occurring about the same time as *Alissonotum impressicolle.* Their number however has always been few as will be evident from the collections noted below. Their life-history is not fully known.

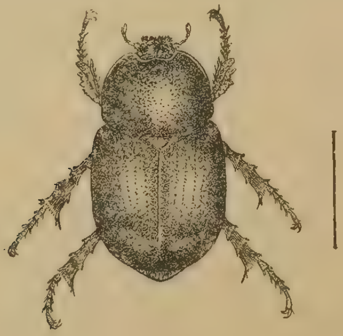


FIG. 3. *Alissonotum crassum*

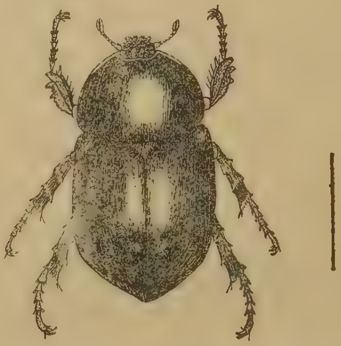


FIG. 4. *Heteronychus robustus*

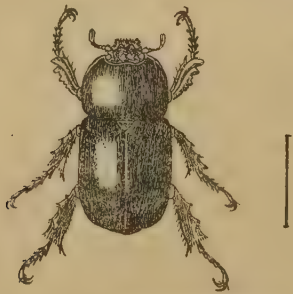


FIG. 5. *Heteronychus liodercs*

In a collection made on 5th May 1927 there were—

Alissonotum impressicolle	10,000
Alissonotum crassum (Fig. 3)	4
Heteronychus sublaevis	1
Heteronychus robustus (Fig. 4)	4

In a collection made on 28th October 1931 there were—

Alissonotum impressicolle	1,000
Heteronychus sublaevis	4

In a collection made in April and May 1934—

Alissonotum impressicolle	78,082
Alissonotum crassum	335

Heteronychus liodereS (Fig. 5) was observed for the first time at Sahmaw and Hopin in April 1933. All the ten specimens collected proved to be female. Unlike all the rest it was observed to take to wing easily and fly from place to place. Its number was however not large to cause alarm.

REFERENCES

Ghosh, C. C. (1921). *Rept. Proceedings Fourth Ent. Meeting, Pusa* (also published as *Pusa Bulletin* No. **134**).

Reports of the Entomologist, Burma, for 1927, 1928 and 1932 to 1935.

Scientific Reports of the Agricultural Research Institute, Pusa, 1918-19 and 1919-20.

MYCOLOGICAL NOTE

UROMYCES RUMICIS (SCHUM.) WINTER : A NEW RECORD FOR INDIA

BY

T. B. LAL, B.Sc. (Agri.)

Post-Graduate Student, Mycological Section, Imperial Agricultural Research Institute, New Delhi

(Received for publication on 5th August 1937)

A species of *Rumex* suffering from a rust was collected at Karnal in April 1937. The affected leaves were full of minute sori which were amphigenous but occurred in larger numbers on the upper than on the lower side. The sori were deep or light brown in colour. They were roundish, and less than half a millimeter in diameter. The host was identified as *Rumex dentatus* Linn. at the Royal Botanic Gardens, Calcutta.

Sections of the leaves and scrapings of the sori mounted in lactophenol showed under the microscope two kinds of spores, one a thin-walled and the other a thick-walled variety. It was easy to identify the former as uredospores and the latter as teleutospores. The uredospores were ellipsoidal with a bright brown, irregularly and sparsely spiny wall with predominantly three (varying from two to four) germ-pores. The teleutospores were nearly globose or invertedly egg-shaped with a thick, deep brown and smooth wall. On the apex of the teleutospore was a semispherical colourless papilla with a single germ-pore. The stalk was colourless. The teleutospores were relatively few in number and the uredospores predominated. One hundred each of the uredospores and teleutospores were measured and the range and mean of the length and breadth are given below :—

	Range	Mean
Uredospores	21-29×17-25μ	25·6×22·0μ
Teleutospores	21-34×17-25μ	24·4×23·4μ

The rust undoubtedly belongs to the genus *Uromyces* of the family Pucciniaceae.

The genus *Uromyces* was founded by Link in 1816 with individually arising unicellular teleutospores. While Fischer [1904], Sydow [1910] and Dietel [1928] retain the genus, Arthur [1934] merges it into the genus *Puccinia* with which it conforms in generic and specific characters. Arthur retains the name *Uromyces*, however, as a matter of convenience to designate those species which have one-celled teleutospores. Seven species of *Uromyces* have so far been reported to occur on the genus Rumex. The shape and colour of the uredospores and the teleutospores, the hemispherical papillæ, the germ-pores and the amphigenous character of the sori clearly place the Karnal species in *Uromyces Rumicis* (Schum.) Winter. The uredo and the teleutospore measurements further confirm this identification for they agree with those given by Fischer [1904], Grove [1913] and Sydow [1910].

I have to thank Dr. B. B. Mundkur for his kindness in helping me with the literature and preparing the manuscript.

REFERENCES

Arthur, J. C. (1934). " Manual of Rusts in United States and Canada ", Lafayette, U. S. A. Page 100.

Dietel, M. (1928). " In Engler and Prantl. Die Naturlichen Pflanzenfamilien ", Leipzig. Page 80.

Fischer, Ed. (1904). " Die Uredineen der Schweiz ", Bern. Page 9.

Grove, W. B. (1913). " The British Rust Fungi ", Cambridge. Page 114.

Sydow, H. (1909-10). " Monographia Uredinearum ", Vol. II, page 238.

NOTE

LENGTH REGULARITY AND PERCENTAGE OF SHORT FIBRES IN COTTON

Sometime ago a new Stapling Apparatus was designed at the Indian Central Cotton Committee Technological Laboratory, with which it is possible to determine the mean length and the fibre-weight per inch (which measures the fineness of staple) of a cotton accurately and quickly. A full account of this apparatus was published in the Journal of the Textile Institute, Manchester. The apparatus is now used in several mofussil stations in India. This apparatus did not yield information regarding the proportion of fibres of different lengths present in a sample of cotton. In certain operations a knowledge of this feature of cotton is important both to the practical spinner and to the research worker. In order to overcome this limitation a new attachment has been designed by Messrs. Ahmad and Nanjundayya—the designers of the Stapling Apparatus—which can be fitted to the Stapling Apparatus and by means of which it is possible to determine the proportion of fibres of different lengths in a sample of cotton. It is also possible with this device to determine quickly the percentage of fibres shorter than a prescribed limit present in a sample. Both these determinations can be carried out quickly and smoothly and tests at the Laboratory have shown that the attachment gives consistent results which agree well with other standard methods. A full account of it will be published in due course.

REVIEW

Proceedings of the Association of Applied Biologists, October 1936. University Press, Cambridge. Price 1*s*. 7*d*.

A GENERAL meeting of the Association of Applied Biologists was held in the Imperial College of Science and Technology, London, on Friday the 9th October 1936, under the presidency of Dr. J. Henderson Smith. The subject of the agenda was "The Problems Raised by the Wooly Aphis (*Eriosoma lanigerum* Hausm) of the Apple—a Case for Team Research" by members of the staff of the East Malling Research Station and the John Innes Horticultural Institution. In his introductory remarks Dr. R. G. Hatton of the East Malling Research Station pointed out that the practical problem which presented itself was the control of Wooly Aphis in nursery and orchard. Since there were difficulties in the way of the usual methods of attack, the use and value of immune rootstocks called for investigation. Wooly Aphis was serious in England from two aspects. It reached epidemic proportions in orchards especially in certain valuable varieties of apple when environmental conditions were favourable and the primary damage caused to new growth and buds was likely to be followed by fungus troubles such as ordinary apple canker. Although nobody cares to admit that he has Wooly Aphis in his nursery, the pest is a continual trouble not only on the rootstock propagation beds but on the buds and grafts and even on the subsequent young trees which are made unsightly and unsaleable. The pest is of even greater importance in the orchards of many overseas apple-growing areas, especially within the British Commonwealth. The almost universal adoption of the use of immune rootstocks in Australia, New Zealand and South Africa bears witness to the seriousness with which the problem was regarded. It was Theobald who pointed out for the first time the necessity of the use of Northern Spy as stock for the eradication of Wooly Aphis. He asserted that until immune stock was found, this pest was sure to occur again and again however well the trees may be sprayed. Theobald's claim about the susceptibility of other rootstocks and the immunity of Northern Spy as a stock for European varieties under English conditions was most stimulating to a wide circle of workers especially to the little group of workers at East Malling who had started upon a thorough investigation of stock-scion relationships. Moreover, from a theoretical point of view, Northern Spy—(difficult to propagate vegetatively, reputedly surface rooting and poorly anchored, varying in behaviour with different scion varieties and under different environmental conditions, with its almost unique degree of immunity to Wooly Aphis)—offered a tempting problem to the team of pomologists, physiologists, propagators, chemists, entomologists, geneticists, and plant breeders who were gathered together in an attempt to measure and explain the rootstock-scion relationships, to improve economic practice and to increase knowledge of fruit plant behaviour. Evidence has been forthcoming that the immune Northern Spy was by no means universally satisfactory as a rootstock. This fact naturally led on both to the study of the possible causes of its lack of efficiency and also to the search for other immune rootstocks. Since the field of choice was very limited, the breeding of new immune varieties offered the most hopeful alternative, and for this end it was necessary to devise a technique for testing seedlings in quantity for their immunity. This might possibly be approached not only entomologically but from a chemical or physiological standpoint. The need then arose to test these new immune rootstocks for their horticultural value in the nursery and orchard. Meanwhile theoretical considerations were cropping up at every turn. Although it has been impossible to present all the available information in these proceedings, the important developments in the present stage of these investigations are outlined in the following seven papers given in the resumé.

II. The Northern Spy as a Rootstock. By R. G. Hatton, C.B.E., M.A. (Oxon.), D.Sc. (Lond.), V.M.H.

III. The Root System of Northern Spy. By W. S. Rogers, M.A., Dip. Hort. (Cantab).

IV. The Control of Wooly Aphis. By R. M. Greenslade, B.Sc., A.R.C.S.

V. Breeding Immune Rootstocks. By M. B. Crane.

VI. Entomological Technique. By A. M. Massee, D.Sc. (Lond.), F.R.E.S.

VII. Pomological Selection of the New Rootstocks. By H. M. Tydeman.

VIII. Studies on Possible Causes of Immunity. By W. A. Roach, D.Sc. (Lond.), A.I.C.

II. The Northern Spy as a Rootstock

When towards the end of the 19th century Northern Spy was being hailed as the salvation of apple orcharding in Australia, New Zealand and South Africa, because of its root and stem immunity to Wooly Aphis, no accurate data were available upon the question of stock-scion relationships in general. The rootstock was regarded merely as a medium upon which varieties which did not themselves root easily could be budded or grafted. Hence, the entomological considerations of immunity to Wooly Aphis were chiefly responsible for the introduction of Northern Spy as a rootstock. Later, when the question of rootstock-scion relationships came to be investigated and comparative tests were made between trees on Northern Spy and other rootstock, doubts with regard to the value of the former as a rootstock were expressed. Two small field-experiments on bush and half-standard trees of the variety Grenadier worked upon Spy and other clonal rootstocks were laid out. At the end of fifteen years the trees on Spy were found to be strikingly less vigorous than those on a number of vigorous and very vigorous layered rootstocks. Summer growth census analyses also revealed differences. The trees on Spy showed typical foliage symptoms of potassium deficiency and were stunted in growth, whilst the trees on Spy were, with the exception of those on Malling No. II, the most precocious to blossom and crop, but increased so little in size that they were soon surpassed by trees of most of the other stocks. The fruits of Grenadier on Spy were smaller and yellower in appearance when compared with those on No. XVI. Fruits from trees on Spy No. I and O. F. V. were for three seasons kept for comparison at Long Ashton throughout their whole storage life. Whilst each year the fruits on Spy showed lower percentage of ‘ Rot ’, ‘ Scald ’ and ‘ breakdown ’, this must be considered in relation to relative maturity at picking. Seasonal effects were much greater than the stock effect. Evidence in literature and unpublished suggested that these peculiarities were not confined to the variety Grenadier under the conditions at East Malling. Complaints of the unsuitability of Spy were frequent. The writer's personal observations in Tasmania, Victoria, Queensland, New Zealand and South Africa confirmed the uncertain behaviour of Spy as a stock and proved that conditions other than environmental were the cause of stunted orchards. Trees on seedling rootstocks were seen to thrive where those on Spy failed. The practical ‘ inarching ’ experiment in Tasmania where seedling stocks were introduced into the stems of the stunted trees on Spy strongly suggested that a change of the root system could completely re-invigorate trees *in situ*.

III. The Root System of Northern Spy

It was suspected for long that the distinctive behaviour of trees worked upon Northern Spy might be largely or partly due to the root characteristics of this stock. To throw further light on this matter the root excavation technique already developed at East Malling was used in a number of Northern Spy trees and stocks ranging from two to seventeen years old, together with comparable material on certain Malling standardized rootstocks. The full details of the work have been reported elsewhere but this paper is intended merely to give an outline of the results so far obtained to show how they fit in with the other studies and to demonstrate the possibilities of the technique. Some forty-seven trees of Northern Spy and comparable stocks were excavated in detail. The root system of Northern Spy was found to be distinctive in that it was usually sparse, one-sided, lacking in fibre, and only tending to send down deep roots at the extremities of the root system. Root competition or other soil factors may make the root system of Northern Spy occupy better layers of soil but these roots did not appear to flourish. The paper has four figures illustrating growth of trees and one root plan of a fifteen-year-old Grenadier on Northern Spy.

IV. The Control of Wooly Aphis

The Wooly Aphis was originally an inhabitant of the eastern side of North America and was introduced into England about 1787 with American nursery material. Since that time it has spread to almost every apple-growing country in the world. It is almost confined to the apple, being very occasionally found on other plants and is a stem and root feeder. The feeding of the Aphides gives rise to galls which may stunt or even kill the young growth of nursery trees and which split and provide an entry for canker fungi on older trees. Even slight galling renders nursery material unsaleable, and the galled areas are particularly susceptible to winter injury by extreme cold. Since Wooly Aphis first became a serious pest of the apple attempts have been made to control it by all the ordinary orchard methods including the use of resistant varieties as rootstocks. The numerous methods which have been tried or suggested reflect the great difficulty of controlling Wooly Aphis. There are many reasons why Wooly Aphis is difficult to control and they are briefly outlined in this note. The author describes in detail the type of damage caused by it. Various methods used for its control such as spraying, dusting, hand painting, trapping, fumigation, tree injection, and biological control, with their relative merits, are fully discussed. Two illustrations showing excavated root systems of young Northern Spy and old Grenadier on Northern Spy are given.

V. Breeding Immune Rootstocks

The breeding experiments with rootstocks and varieties of apples were conducted in the year 1922. The original objects of the investigations were first to obtain a series of rootstocks in which immunity to the Wooly Aphis was combined with other desirable characters, and secondly to attempt to elucidate the mode of inheritance of immunity. The hereditary behaviour of the apple in respect of immunity to attack by the Wooly Aphis is described. Precise knowledge of the frequency of immunity amongst the numerous rootstocks and varieties of apples in cultivation is limited, but general observations indicate that only a small proportion is immune to the attack of the Wooly Aphis, and it has been found that some of the varieties which are commonly reputed to be immune prove to be susceptible when subjected to critical tests. A list of the varieties of apples and rootstocks used in the breeding experiments is given in the paper. It may be recalled that apples are of two kinds—those with thirty-four and those with fifty-one chromosomes, the so-called diploids and triploids respectively. All those that are used extensively in breeding tests were diploids. A genetical analysis is made of families raised from crossing established varieties of apples with a number of forms in general use as rootstocks. The families have been derived from susceptible × susceptible, immune × immune, susceptible × immune and a few small families from selfing immune and susceptible forms. A total of 3,758 seedlings was raised and their susceptibility or immunity was determined. The paper concludes with a very good discussion. The principal results and the conclusions arrived at are summarised as follows:

(1) The susceptible rootstocks and varieties of apples used in this experiment are genetically of different kinds. Some possess factors or genes concerned with the determination of immunity to the Wooly Aphis and some do not.

(2) Amongst the former kind some susceptibles play a larger part in the transmission of immunity than others.

(3) There is a graduation in the inheritance of susceptibility.

(4) All the immunes used are heterozygous.

(5) Some reciprocal crosses give approximately similar, others dissimilar results.

(6) In the cross so far made, Northern Spy has a higher value in respect of the transmission of immunity than any of the other immune varieties used. Collectively, the results of the breeding investigations indicate that the hereditary behaviour of immunity is determined by and dependent upon a certain balance of genetic factors and that is governed by a number of genes the action of which is in part complementary and in part culminative. The breeding work is being continued with a view to testing the validity of the above conclusions.

VI. Entomological Technique

This work was conducted in February 1921 and the author gives a brief account of the methods used for testing apple stocks and seedlings for the resistance to Wooly Aphis from 1921 up to the present time. By making certain modifications in methods of inoculating seedlings from time to time, the methods now adopted are carried on each year as a matter of routine. In 1925 about 100 seedlings could be tested in one season while at the present time approximately 1,000 seedlings are possible to test in a year. It is of interest to note that a number of seedlings which seemed to be immune when tested in natural conditions were found to be susceptible when they were tested a second time under greenhouse conditions. A number of immune seedlings were sent to certain institutions abroad so that their behaviour could be determined under different climatic conditions. A limited number of seedling clones were despatched to Argentine, Canada, India, Morocco, New Zealand, Russia and Queensland. Results of the behaviour of these seedlings in these countries are awaited with interest.

VII. Pomological Selection of the New Rootstocks

Details of the methods by which seedlings immune to Wooly Aphis are tested as to their suitability as rootstocks for apples are described in this paper. The first procedure in dealing with such seedlings has always been to plant them out in the field and to subject them to the method of vegetative propagation known to horticulturists as ‘ stooling ’. From the results of such tests carried on for several years it appears to eliminate a large proportion of the seedlings. The author gives a full description of preliminary propagation tests and details of the rooting capacity of some of the families of immune seedlings are described. Data of the average annual new wood growth, girth of stem, number of blossom trusses, and weight of fruit, of trees of Lane's Prince Albert on three selected seedlings, Merton Nos. 789, 778 and 793 from Malling No. II × Northern Spy are given. It is shown that trees in these three selections differ widely in their degree of productivity during the six years that they have been under observation. While the trees on No. 789 were considerably most vigorous by their sixth year, trees on No. 778 bore, on the average, slightly more fruit than those on No. 789 and very considerably more than those on No. 793. All were much more vigorous than the control trees on Malling No. IX, and in their sixth year bore a large quantity of fruit. Differences in susceptibility to apple mildew and apple scab between the trees on these three rootstocks are also demonstrated. The various data are statistically interpreted. The paper is well illustrated by diagrams and figures.

VIII. Studies on Possible Causes of Immunity

It is pointed that the seedlings that prove to be immune in England cannot be assumed to be so elsewhere, so that they must be tested again when sent overseas. The varieties Delicious and Fameuse found to be resistant in Tasmania were susceptible in New South Wales while Northern Spy seedling × No. 227 although immune in Washington D. C. was found to be susceptible when tested in Tennessee. These and other similar instances that can be given point to the necessity of re-testing of seedlings on arrival in a new territory. Immunity from Wooly Aphis attack does not appear to be explicable in terms of structure of the host. Aphids were reared on artificial media prepared from the barks of immune and susceptible trees. The development of the insects on the media corresponded to their development on living trees in that the number of young produced was smaller, and their life-period was shorter on the immune than on the susceptible. The results suggest that the cause immunity or susceptibility is carried by a chemical substance insoluble in alcohol or ether and only slightly soluble after prolonged boiling in water. The paper gives an illustration of the apparatus which the author used for growing Wooly Aphis in different media. [R. L. S.]

RECENT PUBLICATIONS OF THE IMPERIAL AGRICULTURAL BUREAUX

I. OBTAINABLE FROM THE IMPERIAL BUREAU OF SOIL SCIENCE, ROTHAMSTED EXPERIMENTAL STATION, HARPENDEN, HERTS

Periodical Abstracts

Lists of publications and papers on Soil Science published in the Empire Overseas in—

	Price *s.*	*d.*
1933	1	0
1934	1	0
Soil Research in the British Empire published during 1935	1	0
Lists of Publications relating to Soils and Fertilisers—		
Published monthly, per annum, post free	10	0
Monthly Letters—		
Free to recipients, within the British Empire, of 'Publications relating to Soils and Fertilisers.' Subscription, outside the Empire, per annum	4	0
Recent Developments in Soil Analysis—		
Quarterly Supplement to the above publications. Separate copies each	0	6

Occasional Papers

	s.	*d.*
Technical Communications—		
34. Tropical Soils in relation to Tropical Crops	2	6
Annual Report: For the year 1934-35	0	6
Bibliographies—		
Bibliography on Coffee	2	0
Catalogue of Journals and Periodicals in the Library of Rothamsted Experimental Station	2	0
Special Publication—		
The Katamorphism of Igneous Rocks under Humid Tropical Conditions (by the late Sir J. B. Harrison)	5	0
Bibliography of Soil Science, Fertilizers and General Agronomy, 1931-34	25	0

II. OBTAINABLE FROM THE IMPERIAL BUREAU OF ANIMAL HEALTH, VETERINARY RESEARCH LABORATORY, NEW HAW, WEYBRIDGE, SURREY

Abstracting Journal

	s.	*d.*
The Veterinary Bulletin—		
1931. Vol. 1. Quarterly (1st Number, April)	7	6
Annual Subscription	20	0
Subsequent Volumes. Monthly (1st Number, January)	5	0
Annual Subscription (postage paid)	40	0

Indexing Publication

	Price s.	d.
Index Veterinarius—Four issues a year. First issue April 1933—Annual Subscription (postage paid). Volumes I to III mimeographed, Volume IV onwards printed	100	0

III. OBTAINABLE FROM THE IMPERIAL BUREAU OF ANIMAL NUTRITION, ROWETT RESEARCH INSTITUTE, BUCKSBURN, ABERDEEN

Journal

Nutrition Abstracts and Reviews. (Issued under the direction of the Imperial Agricultural Bureaux Council, the Medical Research Council and the Reid Library)—

	s.	d.
Subscription per volume of 4 numbers	42	0
Per single number	13	0

Occasional Papers

Technical Communications—

	s.	d.
7. Wheat: Pre-eminence as a Cereal Food, Nutritive Value; Relation to Health and Disease	1	0

Occasional Communications—

1. The Effect of Climate on the Composition of Pasture Plants.

IV. OBTAINABLE FROM THE IMPERIAL BUREAU OF PLANT GENETICS (FOR CROPS OTHER THAN HERBAGE), PLANT BREEDING INSTITUTE, SCHOOL OF AGRICULTURE, CAMBRIDGE

Journal

Plant Breeding Abstracts—

	s.	d.
Quarterly. Annual Subscription	15	0
Single copies	5	0

Occasional Papers

	s.	d.
Indexes to Plant Breeding Abstracts—		
Subject Index to Vols. I to V of Plant Breeding Abstracts	5	0
Supplements to Plant Breeding Abstracts—		
Summary of Reports received from Countries exclusive of the British Empire, 1928-31. Supplement I	2	6
Summary of Reports received from Stations in the British Empire, 1932-1935. Supplement II	5	0
Technical Communications—		
Vernalization and Phasic Development of Plants (Joint Publication of the Imperial Bureaux of Plant Genetics)	10	0
Bibliographical Monographs—		
The Experimental Production of Haploids and Polyploids	5	0
Breeding Resistant Varieties, 1930-33 (Supplement)	2	0
An Outline of Cytological Technique for Plant Breeders	1	6

V. OBTAINABLE FROM THE IMPERIAL BUREAU OF PLANT GENETICS (HERBAGE PLANTS), WELSH PLANT BREEDING STATION, AGRICULTURAL BUILDINGS, ALEXANDRA ROAD, ABERYSTWYTH, WALES

Journals

	Price s.	d.
Herbage Abstracts—		
Quarterly. Annual Subscription	15	0
Single numbers	5	0
Herbage Reviews—		
Subscription is at present—Vol. 1 (1933), Vol. 2 (1934), Vol. 3 (1935), Vol. 4 (1936)—included in that to Herbage Abstracts		

Occasional Papers

	s.	d.
Bulletins—		
18. Pastures and Forage Crops in South Africa	3	0
19. Production of Grass Seed	5	0
20. Insects and other Pests Injurious to the Production of Seed in Herbage and Forage Crops	2	6
21. The Influence of Climatic conditions on Type Composition	1	0
22. Technique of Grass Seed Production at the Welsh Plant Breeding Station	5	0
23. Production of Legume Seed	5	0
24. Collection of Native Grass Seed in the Great Plains, U. S. A.	2	0

VI. OBTAINABLE FROM THE IMPERIAL BUREAU OF FRUIT PRODUCTION, EAST MALLING RESEARCH STATION, EAST MALLING, KENT

Journal

	s.	d.
Horticultural Abstracts—		
A quarterly abstract publication of current horticultural literature—		
Annual Subscription	15	0
Single copy	4	0

Technical Communications

	s.	d.
8. Horticultural Aspects of Woolly Aphis Control together with a Survey of the Literature. R. M. Greenslade	2	6

Occasional Papers

	s.	d.
3. Annotated Bibliography on Bitter-Pit, 1934	1	6
Other Publications—		
Index to Volumes I—X of the *Journal of Pomology and Horticultural Science*, 1933. Compiled by Bureau, published by the Editors of the *Journal of Pomology and Horticultural Science*. Available from the Bureau	5	0
Old and New Standpoints on Senile Degeneration, 1931. By A. P. C. Bijhouwer	0	6

VII. OBTAINABLE FROM THE IMPERIAL BUREAU OF ANIMAL GENETICS, INSTITUTE OF ANIMAL GENETICS, UNIVERSITY OF EDINBURGH, KING'S BUILDINGS, WEST MAINS ROAD, EDINBURGH

Journal

	s.	d.
Animal Breeding Abstracts (Quarterly). Commencing April, 1933. Annual Subscription	15	0

Occasional Papers

	Price s.	d
Animal Breeding in the British Empire. A Survey of Research and Experiment, 1934	2	0

VIII. Obtainable from the Imperial Bureau of Agricultural Parasitology, Institute of Agricultural Parasitology, Winches Farm Drive, Hatfield Road, St. Albans, Herts

Journals

	s.	d
Bibliography of Helminthology. For the year 1933—		
Library Edition, bound in cloth	10	6
Stiff paper cover only	8	0
Helminthological Abstracts. (Issued in five or six parts per volume, each volume covering the literature of a single year.) Subscription per volume, payable in advance	30	0
Completed volumes bound in cloth	32	0

Occasional Papers

	s.	d
The Effects of some Natural Factors on the Second Ecdysis of Nematode Infective Larvae. By G. Lapage. 25 pp.	4	0
Notes and Memoranda—		
11. Recent Developments in the Control of *Heterodera marioni*. 6 pp.	1	6

PUBLICATIONS ISSUED BY THE IMPERIAL INSTITUTE OF ENTOMOLOGY, 41, QUEEN'S GATE, LONDON, S. W. 7

	s.	d
Bulletin of Entomological Research.—Published quarterly and containing original articles on Economic Entomology. Issued post-free. Annual subscription (payable in advance)	30	0

Review of Applied Entomology.—Consisting of abstracts or reviews of all current literature on Economic Entomology throughout the world. Published monthly in two series. Series 'A' dealing with insect and other arthropod pests of cultivated plants, forest trees and stored products of animal and vegetable origin, Series 'B' dealing with insects, ticks, etc., conveying disease or otherwise injurious to man and animals. Issued post-free.

	Series 'A' s.	Series 'B' s.
Annual subscription (payable in advance)	30	15
Vol. XXIII (1935)	42	21

	Price *s.*	 *d.*
Zoological Record—Part Insecta.—Published annually about July in each year and containing as complete a record as possible of the literature of the previous year chiefly from the systematic standpoint.		
Annual subscription (including postage)	15	6
Report of the Fourth Imperial Entomological Conference, 19-27th September 1935	4	0

Publications obtainable from the Imperial Mycological Institute, Kew, Surrey

Journal

Review of Applied Mycology.—Annual subscription, 12 monthly parts, with title page and index (post-free)	21	0
Single part	2	0
Title page and index	3	0
Report on the Third Imperial Mycological Conference, 1934	2	0

PUBLICATIONS OF THE IMPERIAL COUNCIL OF AGRICULTURAL RESEARCH, INDIA

[*Prices are inclusive of packing and Indian postage*]

1. Agriculture and Live-stock in India

ICAR. 1 A bi-monthly journal of agriculture and animal husbandry for the general reader interested in agriculture or live-stock in India or the tropics. (Established 1931.) Published in January, March, May, July, September and November. Prepayable subscription Rs. 6 or 9*s*. 9*d*. per annum. Price per part Rs. 2 or 3*s*. 6*d*. Volumes I to VII complete are available.

2. The Indian Journal of Agricultural Science

ICAR. 5 A bi-monthly scientific journal of agriculture and the allied sciences, mainly devoted to the publication of the results of original research and field experiments. (Established 1931.) Published in February, April, June, August, October and December. Prepayable subscription Rs. 15 or 24*s*. per annum. Price per part Rs. 3 or 5*s*. 3*d*. Volumes I to VII complete are available.

3. The Indian Journal of Veterinary Science and Animal Husbandry

ICAR. 6 A quarterly journal for the publication of scientific matter relating to the health, nutrition and breeding of live-stock. (Established 1931.) Published in March, June, September and December. Prepayable subscription Rs. 6 or 9*s*. 9*d*. Price per part Rs. 2 or 3*s*. 6*d*. Volumes I to VII complete are available.

4. Scientific Monographs of the Imperial Council of Agricultural Research

ICAR. 10-1 No. 1. The Fungi of India, by E. J. Butler, C.I.E., D.Sc., M.B., F.R.S. and G. R. Bisby, Ph.D. (1931). Price Rs. 6-12-0 or 11*s*.

ICAR. 10-2 No. 2. Life-histories of Indian Microlepidoptera, Second Series: Alucitidae (Pterophoridae, Tortricina and Gelechiadae), by T. Bainbrigge Fletcher, R.N., F.L.S., F.E.S., F.Z.S. (1932). Price Rs. 3-4-0 or 5*s*. 6*d*.

ICAR. 10-3 No. 3. The Open-Pan System of White-Sugar Manufacture, by R. C. Srivastava, B.Sc. (1935, 2nd edition). Price Rs. 3-2-0 or 5*s*. 6*d*.

ICAR. 10-4 No. 4. Life-histories of Indian Microlepidoptera: Cosmopterygidae to Neopseustidae, by T. Bainbrigge Fletcher, R.N., F.L.S., F.E.S., F.Z.S. (1933). Price Rs. 4-8-0 or 7*s*. 6*d*.

ICAR. 10-5 No. 5. The Bombay Grasses, by E. J. Blatter, S.J., Ph.D., F.L.S. and C. McCann, F.L.S. Illustrated by R. K. Bhide. Price Rs. 20-12-0 or 32*s*. 6*d*.

ICAR. 10-6	No. 6. Helminth Parasites of the Domesticated Animals in India, by G. D. Bhalerao, M.Sc. Price Rs. 7-12-0 or 13*s.* 3*d.*
ICAR. 10-7	No. 7. Influence of Manures on the Wilt Disease of *Cajanus indicus* Spreng. and the Isolation of Types Resistant to the Disease, by W. McRae, M.A., D.Sc. (Edin.), F.L.S. and F. J. F. Shaw, D.Sc. (Lond.), A.R.C.S., F.L.S. (1933). Price Rs. 2-4-0 or 4*s.* 3*d.*
ICAR. 10-8	No. 8. The Silk Industry of Japan with Notes on Observations in the United States of America, England, France and Italy, by C. C. Ghosh, B.A., F.E.S. (1933). Price Rs. 4 or 6*s.* 9*d.*
ICAR. 10-9	No. 9. Mechanical Cultivation in India. A History of the Large-scale Experiments carried out by Burmah Shell Oil Storage and Distributing Company of India, Ltd., by C. P. G. Wade. Price Rs. 3-14-0 or 6*s.* 6*d.*
ICAR. 10-10	No. 10. The Spotted Boll-worms of Cotton in South Gujarat, by B. P. Deshpande and N. T. Nadkarny. Price Rs. 5-14-0 or 9*s.* 6*d.*
ICAR. 10-11	No. 11. Investigations on the Course and Distribution of the Nerves supplying Levator anguli scapuli and Rhomboideus Muscles and the formation of the Phrenic Nerve in the Ox, with Observations on certain Anatomical Deviations, by H. N. Chelva Ayyangar, G.M.V.C. Price Rs. 4-10 or 7*s.* 9*d.*

5. Miscellaneous Bulletins of the Imperial Council of Agricultural Research

ICAR. 8-1	No. 1. List of Publications on Indian Entomology (1930), by the Imperial Entomologist, Pusa. Price As. 14 or 1*s.* 6*d.*
ICAR. 8-2	No. 2. List of Publications on Indian Entomology (1931), by the Imperial Entomologist, Pusa. Price As. 8 or 10*d.*
ICAR. 8-3	No. 3. List of Publications on Indian Entomology (1932), by the Imperial Entomologist, Pusa. Price As. 12 or 1*s.* 3*d.*
ICAR. 8-4	No. 4. Host Plant Index of Indo-Ceylonese Coccidae, by S. Ramachandran, L.Ag. and T. V. Ramakrishna Ayyar, B.A., Ph.D., F.Z.S. Price Re. 1-10-0 or 2*s.* 9*d.*
ICAR. 8-5	No. 5. List of Publications on Indian Entomology (1933), compiled by the Officiating Imperial Entomologist, Pusa. Price As. 9 or 1*s.*
ICAR. 8-6	No. 6. Bee-keeping, by C. C. Ghosh, B.A., F.E.S. (3rd Edition) Price Re. 1-14-0 or 3*s.* 3*d.*
ICAR. 8-7	No. 7. List of Publications on Indian Entomology (1934), compiled by the Officiating Imperial Entomologist and the Imperial Entomologist, Pusa. Price Re. 1-2-0 or 2*s.*
ICAR. 8-8	No. 8. Selected Clinical Articles, Part I, by G. K. Sharma, and R. L Kaura. Price As. 8 or 10*d.*
ICAR. 8-9	No. 9. Statistical Methods and their Application to Agronomy. A Bibliography, by K. K. Guha Roy. Price Rs. 2-2-0 or 4*s.*
ICAR 8-10	No. 10. Diseases of Sugarcane and Methods for their Control, by L. S. Subramaniam. Price Re. 1-14-0 or 3*s.* 3*d.*
ICAR. 8-11	No. 11. Tables of Standard Errors of Mendelian Ratios, compiled by Swarn Singh Purewal, M.Sc., Ph.D. (Cornell), and P. Krishna Rao, L.Ag. Price As. 12 or 1*s.* 3*d.*
ICAR. 8-12	No. 12. List of Publications on the Botany of Indian Crops, Part II, for the Period 1928-32, by R. D. Bose. Price Rs. 3-6 or 5*s.* 9*d.*

ICAR. 8-13	No. 13. Two New Statistical Tbales based upon Fisher's '*t*', by M. Vaidyanathan. Price As. 6 or 8*d*.
ICAR. 8-14	No. 14. List of Publications on Indian Entomology (1935), compiled by the Imperial Entomologist, Pusa. Price Re. 1-4 or 2*s*.
ICAR. 8-15	No. 15. Selected Clinical Articles, Part II, by G. K. Sharma, R. L. Kaura, S. Ganapathy Iyer, G. S. Khan and S. Mangrulkar. Price Re. 1-4 or 2*s*.
ICAR. 8-16	No. 16. Indian Grazing Conditions and the Mineral Contents of some Indian Fodders, by P. E. Lander, M.A., D.Sc. F.I.C., I.A.S. (*In Press*).

6. Annual Report of the Imperial Council of Agricultural Research

ICAR. 12-31	Annual Report of the Imperial Council of Agricultural Research for the years 1929-30 and 1930-31. Price As. 12 or 1*s*. 3*d*.
ICAR. 12-32	Annual Report of the Imperial Council of Agricultural Research for the year 1931-32. Price As. 6 or 8*d*.
ICAR. 12-33	Annual Report of the Imperial Council of Agricultural Research for the year 1932-33. Price As. 6 or 8*d*. (Out of stock).
ICAR. 12-34	Annual Report of the Imperial Council of Agricultural Research for the year 1933-34. Price Re. 1-8 or 2*s*. 6*d*.
ICAR. 12-35	Annual Report of the Imperial Council of Agricultural Research for the year 1934-35. Price Re. 1 or 1*s*. 9*d*.
ICAR. 12-36	Annual Report of the Imperial Council of Agricultural Research for the year 1935-36. Price As. 14 or 1*s*. 6*d*.
ICAR. 12-37	Annual Report of the Imperial Council of Agricultural Research for the year 1936-37. Price Re. 1-2 or 2*s*.

7. Agriculture and Animal Husbandry in India (called Review of Agricultural Operations in India up to 1933)

ICAR. 9-29	Review of Agricultural Operations in India, 1928-29. Price Rs. 3-2-0 or 5*s*. 6*d*. (Out of stock).
ICAR. 9-31	Review of Agricultural Operations in India, 1929-31. Price Rs. 5 or 8*s*. 3*d*.
ICAR. 9-33	Review of Agricultural Operations in India, 1931-33. Price Rs. 5-12-0 or 9*s*. 6*d*.
	Agriculture and Animal Husbandry in India, 1933-34 and 1934-35.—
ICAR. 13-1-35	Part I.—Crop Production. Price Rs. 4-14 or 8*s*.
ICAR. 13-2-35	Part II.—Animal Husbandry. Price Re. 1 or 1*s*. 9*d*.

8. Proceedings of the Board of Agriculture and Animal Husbandry

ICAR. 2	Proceedings of the First Meeting of the Animal Husbandry Wing of the Board of Agriculture and Animal Husbandry held at New Delhi from the 20th to 23rd February 1933, with Appendices. Price Rs. 5-14-0 or 9*s*. 6*d*.
ICAR. 3	Proceedings of the First Meeting of the Crops and Soils Wing of the Board of Agriculture and Animal Husbandry in India held at Delhi from the 25th February to the 2nd March 1935, with Appendices. Price Rs. 6 or 9*s*. 9*d*.

VRI. I	A Description of the Imperial Institute of Veterinary Research, Muktesar, and its Substation, the Imperial Veterinary Serum Institute, Izatnagar, by F. Ware, F.R.C.V.S. Price Re. 1-4-0 or 2*s*.
ARI. 7-187	The Production of Cigarette Tobacco by Flue-curing, by F. J. F. Shaw, C.I.E., D.Sc,, A.R.C.S., F.L.S. and Kashi Ram. *Imp. Inst. Agri. Res. Pusa. Bull.* No. 187. Reprinted (1935). Price Re. 1 or 1*s*. 9*d*.
ICAR. 16	A Handbook of Statistics for Use in Plant Breeding and Agricultura Problems, by F. J. F. Shaw, C.I.E., D.Sc., A.R.C.S., F.L.S. Price Rs. 4-6-0 or 7*s*. 3*d*.

Copies of the above publications can be had from—

The Manager of Publications, Civil Lines, Delhi, and from any of the Agents, a list of whom will be found on the inside page of the front cover.

Purchasers not residing in Asia should apply to the High Commissioner for India, India House, Aldwych, London.

NOTE.—When indenting please give only the symbol preceding the name of the publication.

GIPD—S 3—386 IC of AR—9-12-37—800.